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9537 P

Inhibition of Tissue Respiration by Sodium Benzoate and Sodium Hippurate.

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The consumption of oxygen by minced tissue and by tissue slices in the presence of sodium benzoate was studied in order to determine whether this experimental procedure might be used in the investiga-

* These experiments were performed in the Department of Biochemistry, Oxford, with the generous cooperation and aid of Professor R. A. Peters.

tion of the *in vitro* synthesis of hippuric acid. The oxygen uptake of the tissue was measured at 38°C. in an atmosphere of oxygen using the Barcroft differential manometer. The tissue was suspended in the isotonic salt solution of Krebs and Henseleit[1] with and without the addition of extra phosphate. Sodium benzoate or hippurate, equal to 5 mg. of benzoic acid per cc., replaced an equivalent amount of sodium chloride in the experimental solutions. In most cases glucose was added as a substrate.

Sodium benzoate decreased the oxygen uptake of the following tissues of the rat: liver and kidney slices, diaphragm, and minced brain, liver and kidney. A similar effect was obtained with minced pigeon brain. The inhibition of respiration varied with the concentration of benzoate. It was always more marked if glucose was used as a substrate in a poorly buffered medium due to the production of acid and the resulting decrease in pH. By using tissues, such as minced brain or washed minced liver, which possessed low respiratory activity in the absence of added substrate, it was possible to demonstrate that the inhibitory effect was on the glucose or lactate respiratory mechanism rather than on the succinate mechanism. With minced brain the inhibition was only slightly evident in the presence of succinate and marked in the presence of glucose, lactate or pyruvate. Benzoate toxicity in the presence of pyruvate was unaffected by the addition of vitamin B. Jowett and Quastel[2] have reported that concentrations of benzoate smaller than those used in these experiments affected that respiratory process in liver concerned with the oxidation of fatty acids to aceto acetic acid.

Sodium hippurate resembled sodium benzoate in its inhibition of respiration. It was somewhat less toxic for kidney tissue but was equally toxic for the other tissues listed above, including liver slices with which it has recently been possible to demonstrate the *in vitro* synthesis of hippurate.[3] The similarity in the effects of benzoate and hippurate indicated that the synthesis of the latter was not a detoxication as far as the oxygen uptake was concerned.

1 Krebs, H. A., and Henseleit, K., *Z. physiol. Chem.*, 1932, **210**, 33.

2 Jowett, M., and Quastel, J. H., *Biochem. J.*, 1935, **29**, 2143.

3 Griffith, W. H., *Chem. and Ind.*, 1937, **56**, 552.

9538 P

Entry of Duodenal Contents into the Biliary System of the Guinea Pig.

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While injecting a test-meal into the duodenum of guinea pig Higgins and Mann[1] observed that exceptionally some of the material entered into the common bile duct. Burget and Brocklehurst[2] report that they were not able to drive duodenal contents into the intact ampulla of the guinea pig. Neither group of authors stated the degree of pressure, although Higgins and Mann note that the pressure employed was not unusual.

In 23 out of 26 guinea pigs, we were successful in driving air or dilute India ink into the gall-bladder and biliary passages and section of the liver lobes frequently revealed air in the finer bile channels. The effective duodenal pressures ranged between 10 and 20 mm. Hg; in 2 pigs pressures of 30 and 40 mm. Hg were necessary. In 10 experiments, an intravenous injection of 0.5 to 2 cc. of epinephrin solution (1:10,000) caused generally the entry of air or dilute ink into the gall bladder and bile passages at duodenal pressure-levels that had been ineffective in the same animal before the injection of epinephrin.

The main details of procedure were as follows: The guinea pigs were chiefly males, weighing an average of 700 gm.; sodium barbital, 300 mg. per kg., subcutaneously; abdomen opened in mid-line from the ensiform cartilage to pubis; the antrum of the stomach was ligated; a cannula was tied into the duodenum and connected with a mercury manometer; a compressible bulb in the manometer-circuit permitted accurate variations in pressure.

The pressure that a normal duodenum could exert when stimulated to contraction by faradic currents or by $BaCl_2$ solutions was 20 mm. of Hg.

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1 Higgins, G. M., and Mann, F. C., *Am. J. Physiol.*, 1926, **78**, 344.

2 Burget, G. E., and Brocklehurst, R. J., *ibid.*, 1927-28, **83**, 587.

9539 P

Rôle of Preganglionic Fibers of First Thoracic Nerve in Sympathetic Innervation of Upper Extremity

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From St. Louis University School of Medicine.

Telford[1] and Smithwick[2] have described operative procedures for sympathetic denervation of the upper extremity by means of which the preganglionic fibers in question from the second thoracic nerve downward are interrupted but the inferior cervical and upper thoracic sympathetic ganglia are left *in situ* and the preganglionic fibers of the first thoracic nerve and the gray communicating rami joining the nerves of the brachial plexus are left intact. The advantages claimed for these procedures are avoidance of the unwanted effects of extirpation of the inferior cervical and upper thoracic sympathetic ganglia, particularly Horner's syndrome and sensitization of the vascular musculature in the affected area to adrenin.

The assumption that preganglionic components of the first thoracic nerve play no part in the sympathetic innervation of the upper extremity, although in accord with certain early experimental data recorded by Langley,[3] is not supported by certain more recent experimental and clinical observations. In view of the importance of complete functional sympathetic denervation of the upper extremity in the treatment of peripheral vascular disease it has seemed desirable to obtain additional data regarding the distribution of the preganglionic components of the upper thoracic nerves by means of anatomical and physiological experimentation. The anatomical experiments have been carried out on cats; the physiological experiments on both cats and dogs.

In the anatomical experiments, some cats were subjected to unilateral section of the roots of the second and third thoracic nerves proximal to the communicating rami and division of the sympathetic trunk below the level of the third thoracic nerve; others were subjected to unilateral section of the roots of the first thoracic nerve, leaving the sympathetic trunk intact. Preparations of the inferior cervical ganglia, taken after degeneration of the divided fibers, in the first series, showed extensive degeneration of the intercellular

1 Telford, E. D., *Br. J. Surg.*, 1935, **23**, 448.

2 Smithwick, R. H., *Ann. Surg.*, 1936, **104**, 339.

3 Langley, J. N., *Erg. d. Physiol.*, 1903, **2**, 818.

axon complexes except in the area adjacent to the white communicating ramus of the first thoracic nerve. Preparations of the inferior cervical ganglia, in the second series, showed complete degeneration of the major portion of the intercellular axon complexes in the area adjacent to the white communicating ramus of the first thoracic nerve and relatively little degeneration in other parts of the ganglion. The distribution within the ganglia of the axons arising from ganglion cells in the portion adjacent to the white communicating ramus of the first thoracic nerve also indicates that many of them enter gray rami which join constituent nerves of the brachial plexus.

In the physiological experiments the effects on the blood vessels and sweat glands of the upper extremity produced by direct stimulation of the preganglionic fibers of the upper thoracic nerves by means of an induced current were observed. The electrode was applied to the white communicating ramus of the first, second and third thoracic nerves separately, and, in a few instances, to the cut surface of the ventral root of the first thoracic nerve.

Stimulation of the preganglionic fibers of either the first, the second or the third thoracic nerve consistently resulted in activation of the sweat glands in the paw pads and constriction of cutaneous vessels of the foot. The exact distribution of the sweat glands and cutaneous vessels affected by stimulation of the preganglionic components of each nerve separately has not been determined. It is significant, however, that stimulation of the preganglionic fibers of the first thoracic nerve elicited sweating on all parts of the paw pads.

These experimental data indicate clearly that preganglionic components of the first thoracic nerve play a significant rôle in the sympathetic innervation of the forelimbs in the animals used. Comparative anatomical and physiological data furthermore indicate a close correspondence in the distribution of the preganglionic components of the thoracic nerves in the carnivora and man; consequently, complete sympathetic denervation of the upper extremity in man obviously cannot be accomplished by any operative procedure which leaves intact the first thoracic nerve with its communicating ramus and the inferior cervical sympathetic ganglion with the gray rami which connect it with the constituent nerves of the brachial plexus.

9540

Further Studies on the Virus of the 1937 Outbreak of Encephalitis in St. Louis.

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During the present summer a recurrence of epidemic encephalitis has taken place in St. Louis and St. Louis County. Clinically the disease appears similar to that seen in the same area in the summer of 1933. On September 21, 1937, we reported before the St. Louis Medical Society[1] the successful recovery of a strain of the virus of the present outbreak, the original inoculation being made on August 28, 1937. This had been successfully carried through 4 series of white mice. At that time the first virus neutralization test was in progress but the final report was not available. This virus has now been carried through 8 transplantations in mice. Its virulence for mice is practically identical with that of the virus of 1933. Two strains of the 1933 virus were recovered and studied by two of us in the laboratories of Firmin Desloge Hospital,[2] subsequent to the isolation of the virus by Webster and Fite.[3]

We are now able to report the completion of the 3 virus neutraliza-

TABLE I.
Summary of Results of Virus Neutralization Tests.

Virus Dilution	Type of Inoculation	No. of Animals Inoculated	No. of Animals Surviving
1/10,000	1937 Virus + Normal Serum	10	None
	1937 " + Convalescent (1933) Serum	10	4
	1933 " + " (1933) "	3	None
1/100,000	1937 Virus + Normal Serum	10	None
	1937 " + Convalescent (1933) Serum	10	7
	1933 " + " (1933) "	15	11
1/1,000,000	1937 Virus + Normal Serum	10	1
	1937 " + Convalescent (1933) Serum	10	8
	1933 " + " (1933) "	12	8
1/10,000,000	1937 Virus + Normal Serum	10	4
	1937 " + Convalescent (1933) Serum	10	9
	1933 " + " (1933) "	6	6

[1] Broun, G. O., Greutter, J., Muether, R. O., and Casey, A. E., *Weekly Bull. St. Louis Med. Soc.*, 1937, **32**, 40.

[2] Broun, G. O., Collier, W. D., Muether, R. O., *J. Clin. Invest.*, 1934, **13**, 701.

[3] Webster, L. T., and Fite, G. L., *Science*, 1933, **78**, 463.

tion tests demonstrating the degree of protection afforded by the serum of 3 patients who had acute attacks of encephalitis in the summer of 1933 against the virus of the 1937 outbreak.

Virus neutralization tests were carried out on the sera of these same patients during 1935 and 1936 against the 1933 virus. These tests are included for comparison with the present tests.

The results are summarized in Table I.

The significance of these results is to indicate the identity of the virus causing the 1937 summer outbreak of encephalitis with that which caused the epidemic of 1933. It also points to the existence of an endemic focus of this infection in the St. Louis area.

9541

Concentration of Serum Protein Following a Single Severe Hemorrhage in the Fasting Dog.

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Numerous observations have been made on the fall in serum protein immediately following hemorrhage (particularly plasmapheresis), and its subsequent rise. According to one view, this rise is ascribed to regeneration of new serum protein, presumably from amino-acids which were absorbed from the intestinal tract or mobilized from various tissues, notably the liver and muscle. According to another view, the rise of serum protein is due to the direct entrance of protein from a "body store". This "body store" is apparently different from that contained in the various tissue fluids and lymph which furnish a ready source of fluid, but contain only 1% of protein, which is but one-seventh of its concentration in normal blood plasma.

There is general agreement regarding the fall in serum protein which follows a severe hemorrhage. Accompanied as it is by a fall in the red cell count, the process is obviously one of dilution whereby the fluid stores of the body, poor in protein, enter the blood stream in the attempt to restore blood volume back to normal. Once this dilution has occurred, further increases in serum protein concentration are presumably due to the regeneration of new protein or the direct entrance of new protein from some immediately available source, as

mentioned above. However, no inferences regarding the concentration of serum protein should overlook the influence of changes in plasma volume, which occur particularly after severe hemorrhage. Such blood volume changes are due to the fact that the vasomotor system is especially active after severe hemorrhage, resulting in rapid, and often marked, changes in the size of the blood bed. Thus, actual protein may enter the blood stream and yet be masked as far as its concentration is concerned by a coincident vasodilation; conversely, a vasoconstriction may diminish blood volume and result in an increase in the concentration of serum protein, which, in fact, is unchanged as far as the total amount circulating in the plasma is concerned. In reviewing previous observations on the subject, there is some diversity as regards the behavior of serum protein for several hours after the hemorrhage, although there is universal agreement that definite increases occur after 24 hours, particularly when an adequate diet is ingested. Many observers, however, have found evidence of a marked increase of the serum protein concentration even within a few hours following bleeding. Doubtless much of the variation is referable to differences in procedure and the type of animal used; of great importance, too, is the presence or absence of food from which amino-acids may be absorbed. Prominent among these observations are those of Morawitz,[1] Kerr, Hurwitz and Whipple,[2] Smith, Belt and Whipple,[3] Neumann,[4] Stanbury, Warweg and Amberson,[5] and Cutting and Cutter.[6]

The present observations, in contrast to many of those cited above, reveal little or no increase in the serum protein concentration (after the initial drop) for 24 hours following a single severe hemorrhage. After 24 hours, definite increases were found. In each of the experiments, 3.5% of the dog's body weight was bled and the same amount immediately replaced by Ringer's solution. One-half hour was then allowed to elapse for readjustment to take place before the second sample of blood was taken. All dogs were starved not only during the experiment, but for 48 to 72 hours previously, although water was allowed *ad lib.* Serum protein was determined by macro-Kjeldahl titrations and checked on duplicate determinations

[1] Morawitz, P., *Beit. z. chem. physiol. u. path.*, 1906, **7**, 153.

[2] Kerr, W. J., Hurwitz, S. H., and Whipple, G. H., *Am. J. Physiol.*, 1918, **47**, 356.

[3] Smith, H. P., Belt, A. E., and Whipple, G. H., *Am. J. Physiol.*, 1920, **52**, 54.

[4] Neumann, B., *Arch. f. Klin. Chir.*, 1932, **172**, 529.

[5] Stanbury, J. B., Warweg, E., and Amberson, W. R., *Am. J. Physiol.*, 1936, **117**, 230.

[6] Cutting, W. C., and Cutter, R. D., *Am. J. Physiol.*, 1935, **114**, 204.

to within 2%. The observations are summarized in Fig. 1. These observations confirm those reported by the author in these PROCEEDINGS,[7] in which similar experiments were carried out except

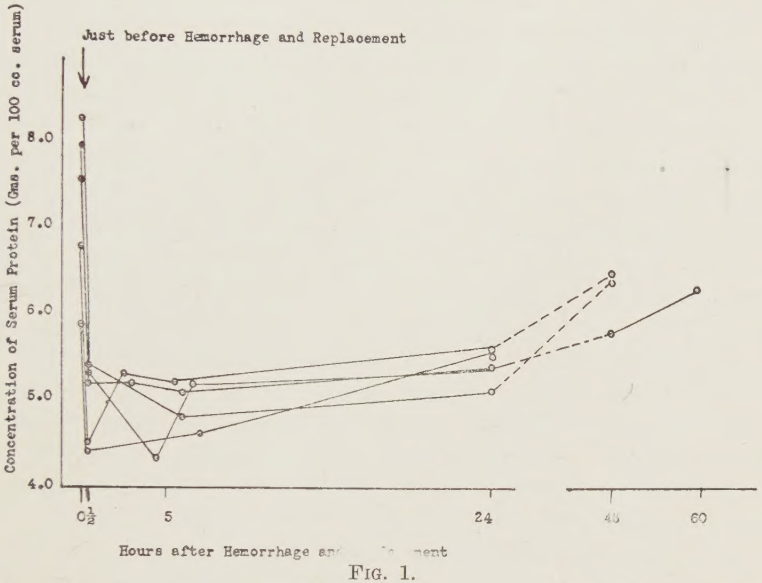

FIG. 1.

Curve of serum protein concentration following a single bleeding of 3.5% of the body weight of the dog, followed by immediate replacement of the same volume of Ringers Solution. Second sample taken at ½ hour.

for the administration of additional fluid following the hemorrhage and replacement. In these experiments there was actually a decrease of 0.09 gm. % in 6 hours with an increase of but 0.15 gm. % in 24 hours following the hemorrhage and replacement. It is of interest to note that Kerr, Hurwitz and Whipple[2] in 1918 made similar observations after plasmapheresis in fasting dogs. They state "Regeneration is a slow and difficult matter requiring many days. . . . It seems that the body can produce serum proteins only in small amounts even in an emergency."

Conclusion. In fasting dogs bled 3.5% of their body weight, with immediate replacement of the same volume with Ringer's solution, very little increase in the serum protein concentration occurred beginning one-half hour up to 24 hours after replacement. After 24 hours, definite increases were noted.

7 Elman, R., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 867.

9542 P

Isolation of St. Louis Encephalitis Virus During Inter and Epidemic Periods.

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In the interval between the 1933 encephalitis epidemic and the present outbreak of the disease, neutralization tests have been made to determine the presence of virucidal antibodies in serum, obtained during convalescence, from 10 patients whose illness had been diagnosed as acute encephalitis. In none of these instances was the test positive. Efforts have also been made to isolate the virus from human brain material obtained at autopsy from 6 fatal cases in which encephalitis was regarded as the cause of death. Intracerebral injections in mice* [1, 2] which proved susceptible in 1933, failed to demonstrate the virus in any of these 6 attempts. Pathological lesions consistent with encephalitis were observed in 3 of these fatal cases, 2 of them being in children.

The clinical diagnosis in the cases without brain lesions might be regarded as incorrect. Concerning those which showed pathological lesions, however, another possibility is that these sporadic cases of the disease were produced by a virus of altered virulence which was not infective for mice in the dilutions employed.

Recalling this interepidemic experience, it was surprising to note the ease with which the virus was isolated from the fatal cases occurring this summer. Using again the same method of intracerebral injection of human brain emulsion into mice, 7 different strains of the virus have already been obtained from 19 different fatal cases. This incidence of the infectivity of brain tissue is almost the same as that observed in 1933 when the virus was first isolated. At that time 7 out of 15 brains were shown to contain active virus by producing the disease when injected into *Macacus rhesus* monkeys.[1] Webster[2] obtained 5 strains out of 11 trials in mice. He was, however,

* Strain C57 Black, first obtained from Dr. Leo Loeb, has been used in this laboratory for the study of encephalitis since 1934. Special strains are apparently not even necessary for the primary isolation of virus from human brain tissue.

1 Muckenfuss, R. S., Armstrong, C., and McCordock, H. A., *Pub. Health Rep.*, U. S. P. H., 1933, **48**, 1341.

2 Webster, L. T., and Fite, G. L., *J. Exp. Med.*, 1934, **61**, 103.

using material from selected cases while in our present experiments every case diagnosed encephalitis was used.

The first strain of virus was isolated on September 8 from material injected September 3. Two other strains were obtained 6 days after inoculation and the remaining 4 on the seventh day.

The results of neutralization tests indicate that the 1937 strains are immunologically similar with those isolated in 1933. Sera from several 1937 patients were tested against the 1933 virus. Tests were also performed using the 1937 virus and 1933 sera. Two samples of 1933 sera were used, a hyperimmune rabbit serum, and serum recently obtained from an individual who survived a severe attack of the disease in 1933. A sample of human convalescent serum stored on ice since 1934 was used in some of the tests, and although originally of high titer it now affords no apparent protection. In each set of neutralization tests, one 1933 positive and 2 negative control sera were used. The dilutions employed were 10^{-3}, 10^{-4}, and 10^{-5}.

Titration experiments to determine the potency of the virus have been carried out on 2 of the recently isolated strains after the fourth passage through mice. One strain is active in dilutions as high as 10^{-5} and the other to only 10^{-4}, when injected in .025 cc. amounts. It is probable that the virulence of these strains will increase after further passage in mice as was the case with the 1933 strains. At present this difference in virulence has remained constant after several additional animal passages. A similar slight but constant difference in virulence has also been observed among 4 strains of 1933 virus maintained in this laboratory.

The virulence of the two 1937 strains studied does not, however, differ greatly from that previously observed here and reported by Webster.[3, 4] Using an already established virus in unselected stock mice, Webster reports an infectivity at varying levels between 10^{-4} and 10^{-6}, when injected in 0.03 cc. amounts. With selected Swiss mice the titer was higher, namely, 0.03 cc. of a dilution of 10^{-7}. The variation in virulence of different strains of the virus isolated during the two epidemics is now being studied.

So far the virus has not been demonstrated in nasal washings using broth, pH 7.6, nor in the spinal fluid from active cases. Guinea pigs have been injected intracerebrally and intraperitoneally with brain tissue and spinal fluid removed early in the disease to test for the virus of choriomeningitis, without positive results. Sufficient time has not yet elapsed since some of these animals were injected to be sure of negative results.

3 Webster, L. T., Fite, G. L., and Clow, A. D., *J. Exp. Med.*, 1935, **62**, 827.

4 Webster, L. T., and Fite, G. L., *J. Exp. Med.*, 1934, **61**, 411.

The pathological lesions observed in the brains of fatal human cases are identical with those described in the previous epidemics.[5, 6] The changes produced in mice by the 1937 strains are also the same as those reported by Smadel and Moore[7] in the case of the 1933 virus.

9543 P

Photoelectric Plethysmography of the Nasal Septum in Man.

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Advantage has been taken of the fact that the opacity of tissues to light varies with the blood content, to record photoelectrically the changes in the blood content of the nasal septum.

The arrangement used (shown schematically in Fig. 1) is essentially an adaptation of the photoelectric plethysmograph for the finger, previously reported.[1] Illumination of the septum is provided by a small ophthalmoscope bulb inserted in one nares. Local heating is largely prevented by a heavy metal cap which, placed over the bulb, conducts the heat away to the lamp carrier. Local heating may be practically eliminated by using a mirror arrangement which permits the light source to be placed some distance from the nares. The latter method of providing illumination has the additional advantage of offering opportunity to control amplification, independent of septal luminosity, through predetermined decrements in light intensity by means of suitable filters. The light transmitted by the septum is reflected out the other nares by the mirror to the photoelectric cell. The entire assembly is mounted on a dental impression plate which, carried between the teeth, not only provides a rigid mount but also guarantees constancy of alignment with the nasal septum. The mirror and light tubes may be varied in diameter to fit varying nares. Any desired penetration is readily provided. The apparatus is light and comfortable and may be worn for hours without discomfort. Breathing through either the mouth or nose is equally feasible. The photoelectric oscillations are recorded galvanometrically on the photokymograph after amplification.

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[5] McCordock, H. A., *Am. J. Public Health*, 1933, **23**, 1148.

[6] McCordock, H. A., Collier, Wm., and Gray, S. H., *J. Am. Med. Assn.*, 1934, **103**, 822.

[7] Smadel, J. E., and Moore, E., *Am. J. Path.*, 1934, **10**, 829.

[1] Hertzman, A. B., and Spealman, C. R., *Am. J. Physiol.*, 1937, **119**, 334.

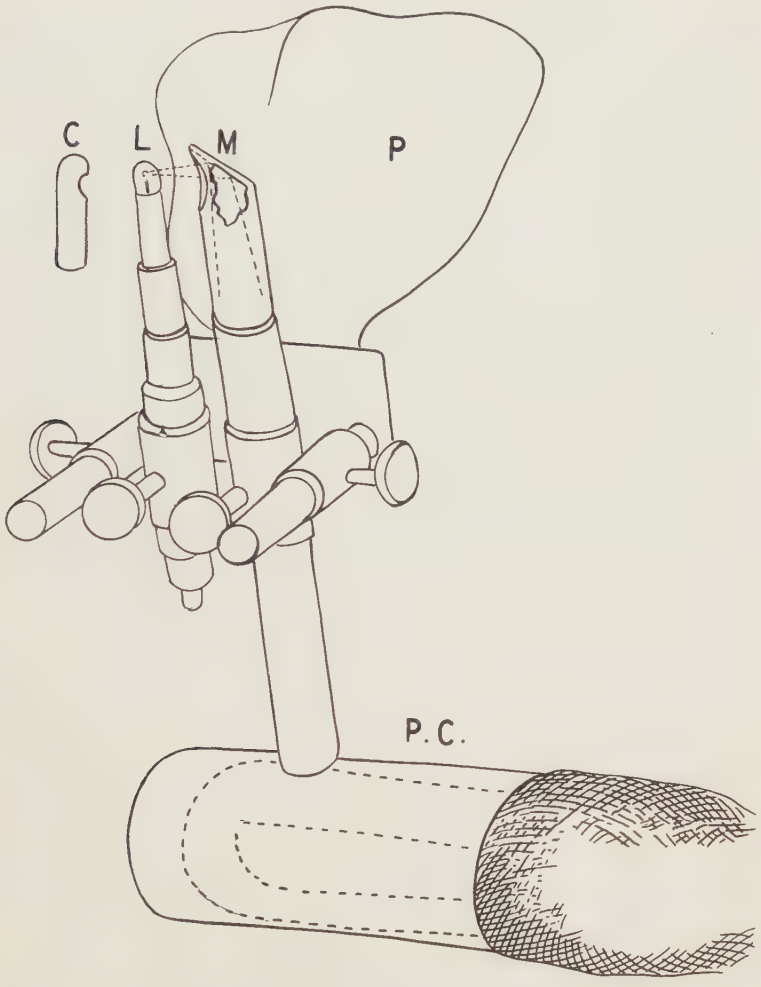

FIG. 1.
Photoelectric plethysmograph for the nasal septum.
M—mirror.
L—ophthalmoscope light bulb.
C—cap for light.
P—dental impression plate.
P.C.—Photoelectric cell housing.

Preliminary observations indicate the feasibility of using the same arrangement for photoelectric plethysmography of the skin of the nose, employing either transmitted or reflected light.

Fig. 2-A shows the volume pulse of the septum so recorded, contrasted with the finger volume pulse (B) of the same subject. Differences in amplitude are in part due to differences in amplification, intensity of transmitted light, etc. The form of the two waves is not

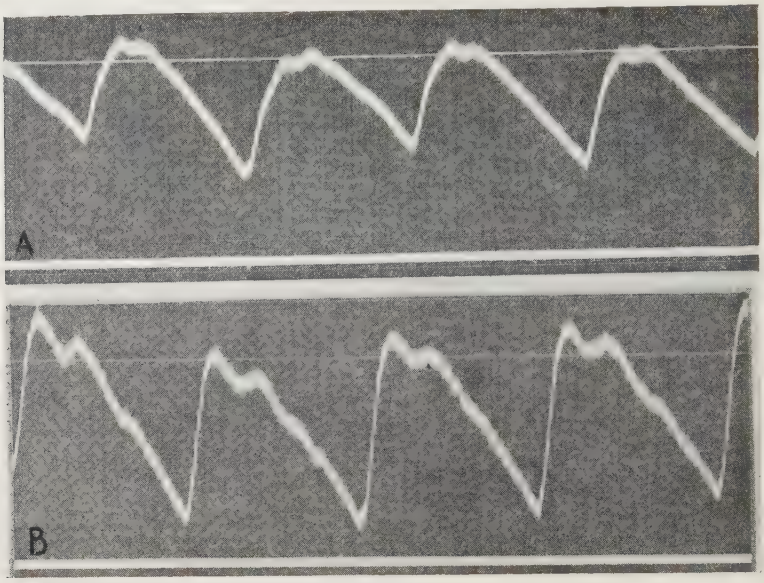

FIG. 2.
Photoelectric plethysmograms.
A—volume pulse of nasal septum.
B—volume pulse of finger—the same subject.

alike; that of the septum is more sustained, more rounded; the dicrotic wave is placed higher and is less pronounced. These differences are regularly encountered in "normal" subjects in this locality although no claim can be made for the "normality" of the septal records so far obtained. It is hoped that the study of wave forms in various circulatory disturbances will provide data for the interpretation of "normal" wave forms.

The apparatus lends itself to prolonged observation on the blood content of the septum although difficulty is experienced due to the movement of the cartilaginous portion of the septum with the passage of air over it. Breathing through the mouth largely obviates this source of error but even an intelligent coöperative subject finds it practically impossible to consistently deviate all the air through the mouth for prolonged periods. Swallowing movements also produce changes in "apparent" blood content. The reporting and interpretation of septal plethysmographic studies is being delayed until these sources of error have been eliminated. They have little significance apparently for the study of wave form. Thus, the septal volume pulse becomes more collapsing in form on the administration of amyl nitrite, suggesting vasodilatation. The opposite change, vasoconstriction, is suggested in the decrease in wave amplitude when the hand is placed in ice-water.

9544

Lactogen Content of Pituitary Glands from Rats on Vitamin Deficient Rations.*

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From the Departments of Dairy Husbandry, Missouri Agricultural Experiment Station and Nebraska Agricultural Experiment Station.

Due to the general influence of the pituitary gland on body function it is important to increase our knowledge of those factors which alter pituitary secretion. Marrian and Parkes[1] reported that vitamin B deficiency, causing loss of body weight or partial inanition, brought about the cessation of estrus and atrophic changes in the ovary. The administration of anterior pituitary substances during the experimental anestrus resulted in the immediate appearance of estrous changes and in ovulation. Evans and Simpson[2] showed that there was a decreased amount of the gonad-stimulating hormone present in the anterior pituitaries taken from animals fed a diet deficient in the antineuritic factor. Mason and Wolfe[3] found an increase in the gonad-stimulating capacity of the anterior pituitaries of male rats which had been fed a diet deficient in vitamin A and which induced xerophthalmia. Furthermore, vitamin E deficiency seemed to have the same effect. Nelson[4] assayed pituitary glands from rats which had been on vitamin E deficient rations and found these glands more potent for the gonad-stimulating hormone than pituitaries from normal animals, but less potent than glands from castrates. Female pituitaries were comparable to glands obtained from normal males. However, Rowlands and Singer[5] reported that vitamin E deficiency caused a definite decrease in the capacity of the pituitary body of the non-pregnant rat to cause ovulation in the estrous rabbit and therefore, a decrease in the content of luteinizing or ovulation-producing substance.

We were interested in determining the lactogen† content of pituitary glands from rats on vitamin deficient rations. Litter-mate rats were paired according to sex and one of each pair fed a vitamin-

* Contribution from the Departments of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 531, and of Nebraska, No. 201.

1 Marrian, G. F., and Parkes, A. S., *Proc. Royal Soc. B*, 1929, **105**, 248.

2 Evans, H. M., and Simpson, M. E., *Anat. Rec.*, 1930, **45**, 216.

3 Mason, K. E., and Wolfe, J. M., *Anat. Rec.*, 1930, **45**, 232.

4 Nelson, W. O., *Anat. Rec.*, 1933, **56**, 241.

5 Rowlands, I. W., and Singer, E., *J. Physiol.*, 1936, **86**, 323.

† In conformity with the usage of estrogen and androgen proposed by the American Medical Association, it is suggested that the term lactogen be applied to the lactogenic hormone.

TABLE I.
Lactogen Content of Pituitary Glands from Rats on Vitamin-deficient Rations.

Vitamin deficiency	No. of animals	Aver. body wt. when sacrificed, gm.	Aver. pituitary wt., mg.	Bird units per pituitary gland	Bird units per mg. pituitary tissue	Bird units per 100 gm. body wt.
A	12*	136	4.5	0.65	0.144	0.48
A	12†	89	3.2	0.40	0.125	0.45
B complex	15*	119	4.5	0.87	0.193	0.73
B complex	15†	41	1.9	0.57	0.300	1.39
D	11*	72	2.4	0.52	0.217	0.72
D	11†	72	2.3	0.36	0.157	0.50
E	18*	285	9.9	1.13	0.114	0.39
E	18†	222	9.7	1.13	0.117	0.51

*Control animals
†Vitamin deficient animals.

deficient ration. The other one of the pair received the same ration plus the vitamin supplement. The rats were fed these rations until vitamin deficiency expressed itself, as determined by the usual methods. Groups of rats were fed rations deficient in the following vitamins: vitamin A, vitamin B complex, vitamin D, and vitamin E. At the end of the experimental feeding period the animals were sacrificed, their pituitaries removed, weighed, and assayed. The glands were assayed by injecting the macerated suspended tissue intradermally over the crop glands of common pigeons, the control pituitary gland over one crop gland and the experimental pituitary gland over the opposite crop gland. (For full details of the technique see paper by Reece and Turner.[6])

The lactogen content of the pituitary glands from rats on vitamin A deficient rations compared favorably with that of the control rats even though they were somewhat lighter in weight. The pituitaries from rats on vitamin B complex deficient rations were much lighter in weight than the pituitaries from control rats. The experimental pituitary glands contained somewhat less lactogen per pituitary gland, but on the basis of equal weight of pituitary tissue the experimental pituitary glands were superior to control pituitaries. Pituitaries from rats on vitamin D deficient rations contained somewhat less lactogen per gland and per milligram of pituitary tissue than pituitaries from control rats. Per pituitary gland and per unit weight of pituitary tissue, the glands from rats on vitamin E deficient rations compared favorably with glands from control rats. The results are summarized in Table I.

[6] Reece, R. P., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bul.*, 1937, 266.

9545 P

A Source of Error in Gonadotropic Hormone Determinations.*

Allan Palmer. (Introduced by C. D. Leake.)

From the Department of Obstetrics and Gynecology, University of California Medical School, San Francisco.

This report is presented in an effort to show that determinations for gonadotropic hormone content of the urine are frequently erroneous due to the precipitation of contaminating amounts of fat-insoluble or combined estrogen.

Concerning urinary gonadotropins there is no doubt that pregnant women excrete a substance capable of producing corpora lutea in the ovaries of normal infantile animals. Some women in the menopause excrete a gonadotropic hormone capable of provoking follicle growth in the ovaries of hypophysectomized rats or mice. A gonadotropic hormone prepared from the pituitary and capable of interstitial cell stimulation has been described, but its detection if present in the urine, depends also upon the use of hypophysectomized animals. For studying the urine of non-pregnant women most workers have resorted to the use of intact animals and depend upon more cursory and unreliable criteria for the estimation of gonadotropic hormone. Speaking collectively, their criteria have been the appearance, in infantile rats or mice as old as 32 days and within periods up to as long as 130 hours after injection, of follicle growth, interstitial cell hypertrophy, increase in uterine and ovarian size and weight, establishment of vaginal introitus, and estrus. The reactions in the lower tubular tract have been interpreted as gonadotropic responses in some instances, in the absence of demonstrable changes in the gonads.

The uterus and its endometrium depend upon the ovaries for changes in histologic structure. If the uterus of an infantile rodent were stimulated directly without the medium of the ovaries but in their presence, by an estrogenic substance that does not in any way depress ovarian activity, then an independent activation of the ovaries by the animal's own pituitary might occur. Hypothetically this pituitary activity can be construed as being either independent and spontaneous, or as a result of stimulation by an estrogen. These are two hypotheses that should be considered in the interpretation of every gonadotropic hormone reaction in intact infantile rodents.

Emmenin is just such an estrogenic substance as described in the

* Supported by the Christine Breon Fund for Medical Research.

foregoing paragraph. Unlike estrone it fails to produce estrus in a castrated mouse in moderate dosage, while in the infantile intact mouse it does cause uterine development and estrus without an apparent depressive action on the ovaries. By hydrolysis I have converted a small amount of emmenin into an active fat-soluble estrogen, a substance that will produce estrus in a castrated mouse in the strength of one International Unit (calculated as estrone) per cc. of emmenin. I have also subjected emmenin to a gonadotropic hormone precipitation procedure, using sodium tungstate, and recovered its active principle.

In a series of tests done on the urine of a normal woman and published elsewhere, I found that the most marked reaction, characterized by estrus, marked uterine enlargement and some evidence of follicle growth, was seen in the animals injected with the gonadotropic hormone concentrate from the same specimen of urine containing the greatest amount of fat-insoluble estrogen during the menstrual cycle studied.

I have done the following experiment in further support of my opening statement. Fifty liters of urine, pooled from the voidings of obstetrical patients, were acidified to pH 5 with H_2SO_4 and treated in the usual manner of precipitation with sodium tungstate. The precipitate was washed many times with 100 volumes of ether and benzene, removing all fat-soluble estrogen. Following this the precipitate was suspended in distilled water, acidified to pH 1 with concentrated HCl, autoclaved for 1½ hours and finally extracted with benzene. The benzene extract was assayed in the usual manner and found to contain a large amount of estrogen.

Conclusion. At least one and possibly other methods of gonadotropic hormone precipitation are effective in recovering fat-insoluble estrogen as a contaminating substance with any gonadotropic substance that may also be present.

9546 P

Sedimentation of Poliomyelitis Virus by Means of a Vacuum Ultracentrifuge.*

Edwin W. Schultz and Sidney Raffel.

From the Department of Bacteriology and Experimental Pathology, Stanford University, California.

This is a brief preliminary report of results indicating that we have been successful in sedimenting poliomyelitis virus, one of the smallest of the ultramicroscopic viruses,[1] from clear aqueous suspensions by means of ultracentrifugation in vacuum. The machine employed is similar in design to that recently described by Bauer and Pickels.[2]

Thus far we have obtained complete results on 2 experiments. In both, the material subjected to ultracentrifugation consisted of a 25% suspension of glycerinated pooled virus cords in physiological saline. The lipoids in these suspensions were removed by ether extraction and the aqueous fraction was centrifuged in an Angle centrifuge at 3000 r.p.m. for at least an hour. In the first experiment the resultant fluid was water-clear to the eye; in the second, very slightly opalescent. Eight cc. of the clear suspension were placed in each of a series of celluloid tubes seated in the rotor.

In the first experiment, the rotor was in motion for a total of 4 hours. Stroboscopic determinations indicated that for 2 hours of this period, the speed was between 27,500 and 30,000 r.p.m. After centrifugation, all of the tubes contained a small membranous type of sediment which could not be completely dispersed by repeated pipetting. The thicker central portion of the sediment presented a pinkish tinge apparently due to sedimentation of hemoglobin present in the original cord suspension.

The supernatant was removed in 2 portions, the upper 6 cc. comprising the "top supernatant," and the pooled bottom 1 cc. portions being the "bottom supernatant." The sediment was resuspended in 6 cc. of saline. Monkeys were injected intracerebrally with 1 cc. quantities of each of these fractions in varying dilutions. The results are presented in Table I.

* These studies were supported by the Mary Hooper Somers Fund for Filterable Virus Research.

1 Clifton, C. E., Schultz, E. W., and Gebhardt, L. P., *J. Bact.*, 1931, **22**, 7; Theiler, M., and Bauer, J. H., *J. Exp. Med.*, 1934, **60**, 767; Elford, W. J., Galloway, I. A., and Perdrau, J. R., *J. Path. and Bact.*, 1935, **40**, 135.

2 Bauer, J. H., and Pickels, E. G., *J. Exp. Med.*, 1936, **64**, 50.

TABLE I.

Monkey No.	Inoculum	Dilution	Results
	Top supernatant:		
C 875	1 cc.	1:400	No poliomyelitis
D 4	1 "	1:800	" "
	Bottom supernatant:		
D 46	1 cc.	1:400	" "
C 874	1 "	1:800	" "
C 871	1 "	1:1600	" "
	Sediment suspension:		
C 930	1 cc.	1:2000*	Poliomyelitis 8th day
C 868	1 "	1:4000*	" " "
C 844	1 "	1:6000*	No poliomyelitis

*Dilutions of the original 6 cc. saline suspension.

In the second experiment, the clear aqueous suspension was subjected to 2 successive runs. In the first run the machine operated a total of 6 hours with a maximum speed of 15,000 r.p.m. for 4¾ hours. At the end of this run the tubes showed no sediment. The centrifuging was therefore continued for another 6 hours, with a speed of 30,000 r.p.m. for a period of about 4½ hours. This time a membranous deposit similar to that observed in the first experiment was found in each of the tubes. The sediment was resuspended in saline equivalent in amount to that used in Exp. 1. Monkeys were injected intracerebrally with dilutions of supernatant and dilutions of sediment as given in Table II.

TABLE II.

Monkey No.	Inoculum	Dilution	Results
	Supernatant:		
D 9	1 cc.	undiluted	No poliomyelitis
D 95	1 "	"	" "
C 632	1 "	1:2000	" "
D 103	1 "	1:2000	" "
	Sediment:		
C 749	1 cc.	1:2000*	" "
D 98	1 "	1:2000*	Poliomyelitis 8th day
C 612	1 "	1:3000*	" 22nd "
D 100	1 "	1:3000*	" 24th "
C 618	1 "	1:4000*	" 7th "
D 97	1 "	1:4000*	" 8th "
D 45	1 "	1:5000*	No poliomyelitis
D 102	1 "	1:5000*	" "
D 55	1 "	1:6000*	" "
D 99	1 "	1:6000*	" "

*Dilutions of the original saline suspension of the sediment.

These results indicate that we have been successful in sedimenting the virus of poliomyelitis from an ether extracted, essentially water-

clear aqueous suspension. It is, however, not yet established that the sedimentation is purely the result of centrifugal force acting on the virus aggregates as such, rather than on aggregates larger than the virus to which it may be absorbed. Further experimental work is in progress.

9547

Studies on Annelid Muscle. II. Observations on Annelid Phosphagen.

ALTON C. KURTZ AND J. MURRAY LUCK.
From Stanford University, California.

That some annelids exhibit peculiarities in the behavior of their phosphagen complex has been demonstrated by Arnold and Luck[1] and by Needham, Needham, Baldwin and Yudkin.[2] This note extends previous observations.

Early in our work on annelid muscle extracts clarified with basic lead acetate we noted positive Jaffe reactions in the lead-free filtrates. More important was the steady increase in the amount of color produced as the filtrates were evaporated at pH 6.0 at temperatures below 100°C. Thus in *Nereis brandti* muscle extract the total "apparent creatinine" values increased 59% when the volume of the extract was reduced to one-fourth the original. Extracts of *Audouinia spirabranchus* muscle gave smaller increases in color production. That the substance responsible for the positive Jaffe reaction was not creatinine itself was shown by negative Weyl and Salkowski tests. The substance could, however, be precipitated by phosphotungstic acid and recovered in the fraction insoluble in absolute methanol. Arginine phosphotungstate is fairly soluble in this reagent, while creatinine phosphotungstate is but slightly soluble.[3] The methanol insoluble phosphotungstate on removal of the precipitant yielded a filtrate giving positive Jaffe and Sakaguchi reactions; attempts to isolate the substance or substances responsible were not successful.

Determinations of labile phosphate in *Audouinia spirabranchus,*

1 Arnold, A., and Luck, J. M., *J. Biol. Chem.*, 1933, **99**, 677.

2 Needham, D. M., Needham, J., Baldwin, E., and Yudkin, J., *Proc. Roy. Soc. London (B)*, 1932, **110**, 260.

3 Drummond, J. C., *Biochem. J.*, 1918, **12**, 5.

Nereis brandti, Glycera rugosa, and also in *Urechis caupo* indicated a slowly-hydrolyzable phosphate, unstable in acid at room temperature. *Glycera rugosa,* the most active species, gave the highest values for this labile phosphorus.

To study further the nature of annelid phosphagen the method of Meyerhof and Lohmann[4] for the isolation of phosphoarginine was applied to the body-wall muscle of *Nereis brandti.* From 160 gm. of muscle there was obtained 0.2 gm. of an impure barium salt. Qualitative tests indicated the presence of barium, phosphate, and some substance giving the Sakaguchi reaction. The phosphorus content was low, indicating that it was possible for only 21% of the isolated material to have been phosphoarginine. Although the Sakaguchi test was positive no trace of arginine could be detected by the use of arginase and xanthydrol. Concentrations of the hydrolyzed barium salt were used which should have given from 25 to 50 times the amount of urea necessary for detection with xanthydrol after treatment with a liver arginase preparation of demonstrated activity.

From these results we conclude that either (a) the procedure used for the isolation was unsatisfactory, or (b), in the light of the cumulative evidence, that the phosphagen of *Nereis* is not arginine phosphate. We favor the latter alternative.

9548

A New Apparatus and an Improved Method for Chromatographic Adsorption.

A. M. POTTS AND F. C. KOCH.

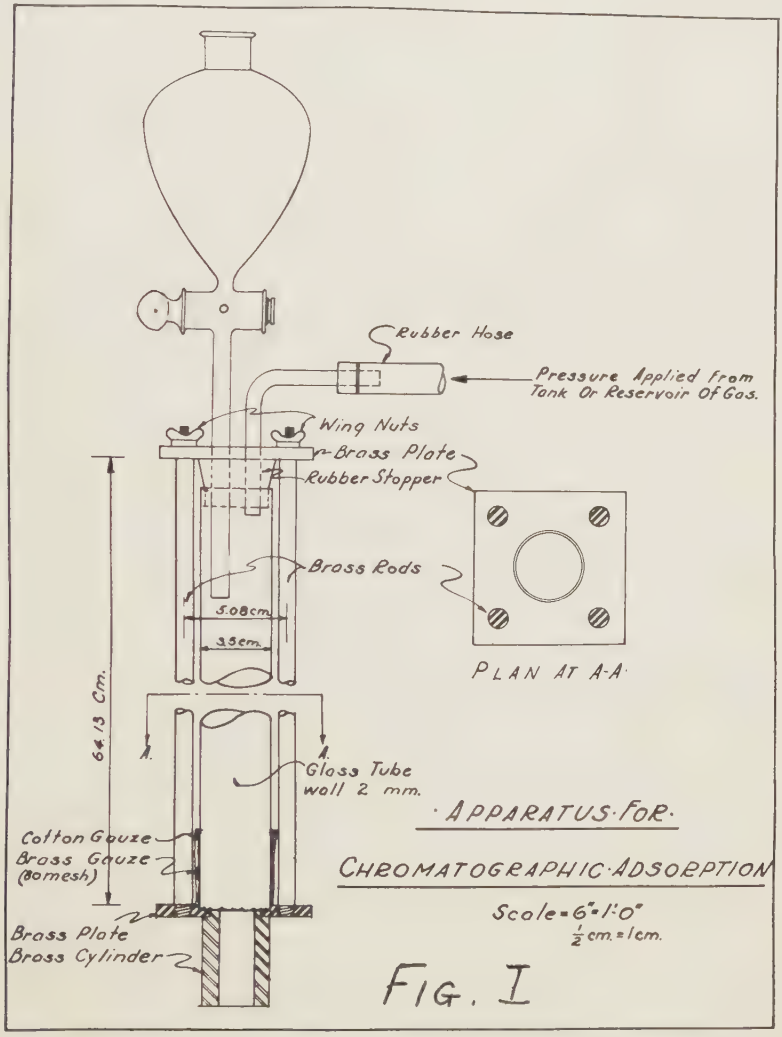
From the Department of Biochemistry, The University of Chicago.

It is well known that chromatographic adsorption has been a very valuable method for the separation of organic compounds of biological importance. The principle involved in the use of our apparatus is essentially the same as in the older forms. The procedure, however, differs in that we filter our solution through the column of adsorbent by pressure and not by suction and we localize the area of specific adsorption of colorless compounds by means of a color reaction carried out on one side of the column of adsorbent or by the fluorescence observed with ultraviolet light. The method pro-

[4] Meyerhof, O., and Lohmann, K., *Biochem. Z.*, 1928, **196**, 49.

duces a more uniform column of the adsorbent; it is more rapid and prevents the irregular results produced by evaporation of the solvent.

Apparatus. The adsorption tube proper (Fig. 1) is a plain piece of Pyrex tubing 3.5 cm. in bore and 60 cm. long. The bottom is covered by a pad of absorbent cotton 1 cm. thick, held in a cup of



brass screening. The screening with cotton under it is wired to the tube to give ease in handling and added protection against leakage of adsorbent. This bottom end rests in the countersunk depression in the lower plate. This plate contains a central outlet hole into

which the outlet tube is screwed and is pierced at the corners by 4 holes into which the rods are screwed.

The top of the adsorption tube is closed during operation by a rubber stopper which is held in against the pressure by the top plate. This plate has 4 holes through which the rods pass. It is held down against the stopper by 4 wing nuts which screw on the ends of the rods. Both the top plate and the stopper have 2 holes at the center through which pass a separatory funnel for admission of the solution and a glass tube for the pressure inlet.

Pressure is obtained from a tank of compressed gas—N_2 or CO_2 is often desirable for easily oxidized compounds. The pressure line also contains a manometer, a drying tube, and a side-tube fitted with a stopcock.

In another modification of the apparatus we provided a reservoir of reasonable capacity below the separatory funnel and above a tube of narrow diameter containing the adsorbent. The reservoir is very convenient in passing larger volumes of solution through the narrow column.

The proper amount of adsorbent is suspended in the solvent to be used until the paste first formed gives way to a slow flowing suspension and this material is poured into the absorption tube. The stopper is inserted in the top and the plate screwed down. The pressure is raised and the excess solvent is forced through. Usually a pressure of one atmosphere is sufficient to give satisfactory packing. When the line of solvent just reaches the level of the top of the adsorbent, pressure is released by opening the stopcock in the pressure line. The stopper and top plate are removed and a pad of glass wool is introduced into the top of the column. A cork of the same diameter as the column is inserted and the packing is completed by applying pressure on the cork with a piece of dowel pin. The cork is then removed by means of a wire previously attached for the purpose. The glass wool is left to keep the top layer of adsorbent from being stirred up by the addition of liquid.

The solution to be adsorbed is poured in, the stopper and plate are replaced, and the pressure is raised. When the solution has passed through, the pressure is released, the developer solution is added through the separatory funnel, and the pressure is raised again. In the experiments conducted so far, the volume of the empty tube over the adsorbent has been about 200 cc., and since only 600 cc. of developer was used, the release of pressure 3 times was quite feasible. For larger volumes of solution it might be advantageous to have a method of adding liquid without interrupting the

course of adsorption. It would not be difficult to arrange an auxiliary pressure line to the separatory funnel by means of a T-tube, and add liquid from a smaller funnel into the large one. Thus the large separatory funnel could be filled, and a slight increase in pressure would force the liquid into the adsorption tube.

After development is finished (and not before) the liquid is allowed to fall below the top of the adsorbent and all excess solvent is forced out. The tube is removed from the apparatus, the bottom screen is taken off, and the column of adsorbent is pushed out by the cork and dowel pin used previously.

A fresh surface of column is obtained by cutting away a small groove along the whole length of the cylinder, and this surface is observed under ultraviolet light if there are any fluorescent compounds whose location is desired. In the case of a non-fluorescent compound that gives a color reaction, the reagent is dropped along the fresh surface by means of a pipette. The column is again viewed under the ultraviolet lamp because it has been frequently noted that the reaction may create new compounds that fluoresce. It is obvious that a color is not essential here; the reagent need only convert the colorless non-fluorescent compound to a colorless fluorescent one. With the aid of the data thus obtained the bands are cut as desired, the parts contaminated by the reagent are discarded, and each band is eluted separately. Zechmeister, Cholnoky, and Ujhelyi[1, 2] have also detected the location of colorless adsorbed substances by applying a color reaction on the side of the column.

Summary. A new apparatus whose advantages are increased speed and uniformity of column has been described. A new method of working with colorless, non-fluorescent compounds by use of a color reaction has also been reported.

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1 Zechmeister, L. M., Cholnoky, L. V., and Ujhelyi, E., *Bull. Soc. chim. Biol.*, 1936, **18**, 1885.

2 Zechmeister, L., and Cholnoky, L. V., *Die Chromatographische Adsorptionsmethode*, Vienna, 1936, Julius Springer.

9549 P

The Egg as a Medium for Cultivation of Mycobacterium Tuberculosis from Tuberculous Materials.

G. I. Wallace and M. R. Weissbuch. (Introduced by F. W. Tanner.)

From the Department of Bacteriology, University of Illinois.

During the course of a comparative study of media for isolation of *Mycobacterium tuberculosis* from sputum and other infectious material, it was decided to attempt culture of this organism in eggs. This was prompted by the observation that cultural methods now used for this purpose prescribe media containing whole eggs or egg yolk and because of the successful cultivation of viruses and bacteria on the chorioallantoic membrane of the developing egg. The developing egg has been used by Brandly and Graham[1] and Bradford and Tittsler[2] for the culture of bacteria. The former authors obtained growth of Brucella, Salmonella and Pasteurella species upon the chorioallantoic membrane of developing eggs and the latter cultured *Neisseria gonorrhoeæ* in the developing egg. Although these authors were studying the invasive powers or pathogenicity of the inoculated organisms their observations are of interest. Bradford and Tittsler suggest the method as a means of culturing *Neisseria gonorrhoeæ* from sources such as joint fluid.

In the present study the primary object was cultivation of the organism from infected material in the simplest and quickest manner possible. The egg of the chicken was used with no attempt to obtain a developing embryo. As a preliminary experiment, a suspension of a pure culture of freshly isolated *Mycobacterium tuberculosis* was injected into the yolks of fresh eggs. The egg shell was sterilized with 5% phenol followed with 95% alcohol and then pierced with a sterile pin. The suspension of *Mycobacterium tuberculosis* was then inoculated (½ cc.) into the yolk of the egg and the pin hole covered with a drop of paraffin. The egg was then placed in the 37°C. incubator and incubated for 20 days. After incubation the egg was broken and the contents were placed in a sterile Petri dish. One might expect that eggs treated in this manner would be spoiled and quite objectionable but this was found not to be the case. The appearance of the eggs treated in this manner is quite different

[1] Brandly, C. A., and Graham, R., *Science*, 1936, **84**, 315.

[2] Bradford, W. L., and Tittsler, R., Proc. Soc. Exp. Biol. and Med., 1936, **34**, 241.

from that of an uninoculated control egg. The inoculated egg contains numerous, easily visible, greyish patches in both the white and the yolk which are not present in the control. When these greyish patches are picked, crushed upon a glass slide, stained by the acid-fast procedure and examined with the microscope, countless numbers of acid-fast rods are seen in what is apparently a pure culture.

Following this successful culture of *Mycobacterium tuberculosis* in the egg, the procedure was repeated using sputum from a tuberculous patient. In this experiment both fresh eggs and hard-boiled eggs were used and the sputum was concentrated with 6% sulphuric acid, 3% hydrochloric acid, 4% sodium hydroxide, and 5% oxalic acid before inoculation. The eggs were inoculated, using the same procedure previously described, with ½ cc. amounts of the residue after concentration. The results obtained were like those described in the pure-culture inoculations. For example, in one series of 8 raw and 8 hard-boiled eggs inoculated with concentrated sputum 7 of the 8 raw eggs and 6 of the 8 hard-boiled eggs gave very definitely positive growths. In the boiled eggs the greyish patches of discoloration were found mostly in the yolk. If the boiled eggs were inoculated through the air sac the organisms grew in the air sac with the "piled up, crumb like" growth which is so characteristic of *Mycobacterium tuberculosis*. Very seldom could one observe other organisms in the eggs. In the positive eggs the acid-fast organisms were always present in great numbers. Material from positive eggs was inoculated into guinea pigs to establish the pathogenicity of the acid-fast rods. In all cases active tuberculosis was produced in the guinea pigs. In a few cases eggs were examined after 10 days' incubation. Acid-fast organisms could be found in abundance upon microscopic examination but the greyish patches were too small to be easily observed. At the present time it is not known which method of concentration is best for this method of culture.

This procedure gives promise of being a quick and simple method for culturing *Mycobacterium tuberculosis* from tuberculous material.

9550

Production of Nutritional Cataract in Trout.

Walter N. Hess. (Introduced by F. P. Knowlton.)
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In 1931 an investigation showed that, in many of the hatcheries of New York State, practically every trout 6 months of age or older had distinct opacities in both lenses (bilateral cataract). The lenses were not the only organs in the fish that were affected. The different fins, including the tail fin, were abnormal and showed signs of alternating periods of partial degeneration followed by partial regeneration. The iris of the eyes in the older fish showed degenerative changes. The scales likewise showed degenerative changes in these older fish. All fish that had well developed cataracts were dark colored, a condition known to occur in fish that are blind.

This investigation was undertaken to determine whether the cataract in these fish was due to a contagious infection or whether such other factors as diet, light and heredity were the cause.

The methods employed here were much the same as those used in previous work (Hess[1]). Sixteen standard size rearing troughs were used, and were operated at the Caledonia fish hatchery under standard conditions. Fish of 2 different age groups were used, approximately 2½ and 4 months old respectively. There were 2 groups of the younger fish. One (Exps. 4 and 5) were hatched from trout seined from Seneca Lake. The other (Exps. 6, 7, and 8) as well as the older fish used in Exps. 1, 2, and 3 were from hatchery stock. Each trough contained 5000 fish at the beginning of the experiment. Only rainbow trout *(Salmo irideus)* were used.

To test the possibility of contagion 200 trout with cataract were placed for 4 months in a small pond with 400 trout without cataract and fed the control diet of liver and heart.

The older fish at the hatcheries where cataract was so prevalent had been fed exclusively on pig spleen. The fish in the experimental troughs of Exps. 1-7 were therefore given this same diet. In the corresponding control troughs a diet of equal parts of beef liver and beef heart was used. In experiment 8 the fish were fed a mixture of 2 parts of pig spleen and one part of beef liver and beef heart. All fish were fed an abundance of food 6 times daily. It so happened that previous to the experiment the young fish that were used had also been given a diet composed chiefly of pig spleen.

[1] Hess, W. N., *J. Exp. Zool.*, 1935, **70**, 187.

FIG. 1.
Photograph of living rainbow trout showing late stage of cataract. Photographed for the author by G. C. Embody.

In order to test the possible effect of light, some troughs were covered, others were left uncovered, and 2 uncovered troughs were painted white on the inside. All others were painted black. To expose the fish still further to light, the water in some troughs was kept at a depth of 3 inches while in others it was kept at 8 inches.

To test the possibility of a hereditary factor being involved some of the fish (Exps. 4 and 5) were hatched from eggs that had been obtained from trout seined from Seneca Lake. All others were obtained from hatchery trout, a race that had been at the hatchery for years.

Since after 4 months none of the 400 cataract-free trout which had been placed with the 200 trout with cataract had developed cataract, and since histological sections showed no flukes or other parasites in the lenses, it seems clear that the type of cataract under investigation is not of a contagious nature.

In computing the percentage of fish with cataract at the end of the experiment, 200 were taken from each trough and examined under a binocular microscope for evidence of lens opacities.

By consulting Table I it will be seen that cataract appeared in all groups that were fed exclusively on spleen. On the other hand, none of the liver-heart-fed fish, irrespective of other conditions, showed

TABLE I.
Effect of Diet and Light on Development of Cataract in Rainbow Trout.

No. of exp.	No. of trough	Food	Trough covered or uncovered	Depth, water, inches	Av. wt., gm., June 2d	Av. wt., gm., Sept. 2nd	% that survived	% survivors with cataract
1	A	*	covered	8	.64	2.41	82.22	26
	B	†	,,	8	.64	3.89	95.96	0
2	C	*	uncovered	3	.64	2.40	60.69	6
	D	†	,,	3	.64	3.61	71.46	0
3	E	*	,, painted white	3	.64	2.21	13.76	9
	F	†	,, ,, ,,	3	.64	3.18	68.65	0
4	G	*	covered	8	.27	1.52	35.69	31
	H	†	,,	8	.27	2.05	92.96	0
5	I	*	uncovered	3	.27	1.29	27.39	17
	J	†	,,	3	.27	1.84	79.77	0
6	K	*	covered	8	.32	1.49	55.49	11
	L	†	,,	8	.32	3.07	95.55	0
7	M	*	uncovered	8	.32	1.91	56.53	22
	N	†	,,	8	.32	2.61	75.46	0
8	O	2 parts* 1 part†	covered	8	.32	2.58	80.31	0
	P	2 parts* 1 part†	uncovered	3	.32	2.46	71.73	0

*Pig spleen.
†Beef liver and heart.

the least trace of cataract. The older fish were used in Exps. 1-3. Of these, those that were fed pig spleen exclusively showed an average of 13.7% with cataract. The younger fish were used in the other experiments. Of these, those fed on spleen exclusively showed an average of 20.2% with cataract. From this it would seem that the younger the fish are when they are placed on an exclusive diet of spleen, the larger will be the percent of fish that develop cataract in a given time. No opacities appeared in the lenses of fish given the spleen-liver-heart diet (Exp. 8).

Since, as Table I shows, more fish with cataract were found in the covered troughs than in the uncovered troughs, it seems quite clear that exposure to light cannot be considered to be a cause of cataract in these fish.

Since the incidence of cataract was greater among trout obtained from wild stock (Exps. 4 and 5) than among those from hatchery stock, it appears that trout from wild stock are fully as susceptible to cataract as those from hatchery stock.

Hence, we must conclude that cataract in these fish is due to an unbalanced diet. The data above show that a dietary deficiency is responsible rather than any toxic substance since an exclusive spleen diet causes cataract while spleen in combination with other foods does not cause it.

9551

Pancreatic Diabetes in the Rabbit.*

PAUL O. GREELEY. (Introduced by D. R. Drury.) (With the technical assistance of G. Jacobson and M. Kamins.)

From the Department of Physiology, School of Medicine, University of Southern California.

The diffuse distribution of the pancreas in the rabbit and the attending problems of surgical removal have prevented the study of pancreatic diabetes in this animal. In fact, it has been pointed out that "in rabbits the operation itself is impossible, because of the spread-out condition of the pancreas."[1] However, a method for

* We wish to express our sincere appreciation for the generous gifts of insulin from the Eli Lilly Co., and for nembutal from the Abbott Laboratories.

This work was aided by grants for technical assistance from the National Youth Administration.

1 Macleod, J. J. R., *Carbohydrate Metabolism and Insulin*, 1926, p. 78.

complete pancreatectomy is presented here together with data pertaining to insulin requirements, glycosuria, blood sugar levels, etc.

The pancreas in general, is a thin sheet of tissue lying in the mesoduodenum with one large mass on the right between the portal vein and the vena cava and another mass in the mesentery of the spleen.

At operation it is essential to use a Beebe binocular loupe to pick up the small fragments of pancreas which break off and to prevent injury to small intestinal blood vessels. For rabbits of about 2 kilos the anesthetic used is 1 cc. of nembutal intravenously, 0.2 cc. of 1% atropine subcutaneously and sufficient ether at the beginning to allow opening of the abdomen.

Complete removal of the pancreas at one operation usually results in a very high mortality in from 24 to 60 hours from severe diarrhea, gastric detension, surgical shock, or unknown causes. A 3-stage operation with 3-4 weeks intervening between operations gives very satisfactory results. Three different abdominal incisions are used; for the first stage, about 1 inch to the left of the mid-line; for the second, a mid-line, and for the third about 1 inch to the right of the mid-line. Twenty-day chromic catgut size 00 is used for closing the abdomen.

In the first stage, all pancreas in a pocket formed roughly by the transverse colon, stomach and duodenum is removed. A splenectomy may be done by ligating and sectioning the larger vessels, or the pancreas can be gently pulled off from the splenic vessels. The mesentery and pancreatic tissue is removed from the transverse colon, the stomach raised up and the mesentery and pancreas covering the blood vessels carefully pulled free. The small pancreatic blood vessels which are broken stop bleeding in a few minutes. In this stage the gastro-duodenal mesentery is not broken through.

In the second stage all pancreas between the stomach, the inferior pancreatico-duodenal vessels and the vena cava is removed. As the duodenum is held up out of the abdominal cavity, the lateral layer of mesentery and pancreatic tissue is removed. This leaves the medial layer of mesentery to support the blood vessels. Both layers of mesentery may be removed where this support is not essential.

In the third stage, the duodenal loop is freed by cutting a small mesentery attachment which holds the distal portion to the descending colon. The loop is then spread out on a piece of gauze moistened with saline and all the remaining pancreas removed. This is readily accomplished by dissecting free and removing the upper layer of mesentery and pancreas. This prevents the blood vessels supplying the duodenal loop from being injured.

Blood sugars usually rose within a few hours following pancreatectomy.[2] (Table I.) Exceptions occurred in rabbit 9 in which the rise was delayed for 3 days and in rabbit 1 for 9 days. This delayed rise may have been due to incomplete pancreatectomy. At autopsy

TABLE I.
Time of Blood Sugar Increase Following Pancreatectomy.

Date and Time of Pancreatectomy	Date	Blood Sugars Time	mg. %	Date and Time of Pancreatectomy	Date	Blood Sugars Time	mg. %
No. 1	1-11	2:00 P.M.	160	No. 7	4-23	12:15 P.M.	154
1-11-37	1-12	5:00 "	117	4-23-37	4-24	9:30 A.M.	236
11:30 A.M.	1-13	9:30 A.M.	90	12:10 P.M.	4-26	10:00 "	260
	1-15	9:00 "	110		4-27	10:00 "	300
		3:40 P.M.	100				
	1-18	9:00 A.M.	138	No. 8	4-23	4:00 P.M.	154
	1-19	10:00 "	129	4-23-37	4-24	9:30 A.M.	320
	1-20	9:25 "	210	3:50 P.M.			
	1-21	9:30 "	156				
	1-22	10:00 "	266	No. 9	7-28	11:00 A.M.	160
		5:50 P.M.	338	7-28-37		4:30 P.M.	110
				10:30 A.M.		9:00 "	90
No. 2	1-29	6:00 "	133		7-29	10:30 A.M.	105
1-29-37		11:00 "	185			2:40 P.M.	120
5:45 P.M.	1-30	9:00 A.M.	190		7-30	9:30 A.M.	133
		1:50 P.M.	312			4:40 P.M.	222
					7-31	12:30 "	280
No. 3	2-12	9:40 "	245		8- 1	10:10 A.M.	300
2-12-37	2-13	9:00 A.M.	138		8- 4	9:00 "	440
3:00 P.M.		7:15 P.M.	384				
				No. 10	8-24	3:30 P.M.	137
No. 4	3-10	9:00 A.M.	80	8-24-37	8-25	9:30 A.M.	200
3-9-37		5:00 P.M.	133	11:30 A.M.	8-26	3:30 P.M.	400
4:00 P.M.		10:00 "	250				
	3-11	9:00 A.M.	308	No. 11	8-27	11:35 A.M.	172
		1:30 P.M.	396	8-27-37		2:00 P.M.	182
		4:30 "	440	11:30 A.M.		4:00 "	200
					8-28	9:00 A.M.	200
No. 5	4-15	9:00 A.M.	141		8-29	9:30 "	334
4-14-37	4-16	9:30 "	280		8-30	10:30 "	400
		9:00 "	360		9- 1	9:00 "	572
No. 6	4-22	12:05 P.M.	141	No. 12	8-30	3:40 P.M.	200
4-22-37		5:00 "	172	8-30-37	8-31	10:00 "	308
11:30 A.M.	4-23	9:30 A.M.	262	3:30 P.M.	9- 1	9:00 A.M.	400
	4-24	9:30 "	286				

on No. 1, 40 days after pancreatectomy, a piece of abnormal pancreatic tissue was found on the dorsal side of the portal vein. It measured approximately 16 mm. long, 7 mm. wide, and 2 mm. thick, and consisted of a wild proliferation of ducts imbedded in fibrous tissue with an occasional group of cells identified as islets. This rabbit, however, put out as high as 50 gm. of sugar daily.

2 Shapiro, R., and Pincus, G., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 416.

Four of the rabbits presented in Table I lived for extended periods —rabbit 1, 40 days; rabbit 4, 4 months; rabbits 9 and 10 survived for 82 days and 38 days respectively. The other rabbits died in about 3 days from a severe diarrhea or peritonitis.

Blood sugars in the depancreatized fed rabbits without insulin injections remained around 400-500 mg. % as determined by the micro-method of Folin-Wu. The highest amount of sugar excreted in 24 hours occurred in rabbit 4, which put out 54.35 gm. in 1000 cc. of urine. Rabbits 4 and 10 excreted about 5 gm. of sugar daily or were negative with the subcutaneous injection of 6 units of protamine zinc insulin each morning. Rabbit 9 excreted about 1 gm. of sugar daily with 4 units of protamine zinc insulin. The food intake determined the amount of insulin it was possible to give. For example, at one time rabbit 4 received 12 units of protamine zinc insulin daily and still excreted 5 gm. of sugar daily. Good gains in weight were made in each case during the insulin administration.

No acetonuria has been demonstrated by the qualitative test during prolonged periods without insulin.

Pancreatectomy in the rabbit should be as complete as that which is possible in the dog, if each stage of the removal is carefully done. Microscopic examinations of suspected tissue have always been made at autopsy and no pancreas was found except that noted above in rabbit 1. However, rabbits surviving the operation were able to live for long periods without insulin. Rabbit 1 received no insulin for 31 days and maintained a constant weight. A constant weight was also maintained by rabbit 4 for 16 days without insulin. Rabbit 9, however, was kept 34 days without insulin and lost 490 gm. in weight, excreting daily an average of 24 gm. of sugar in the urine. The continued loss of weight despite a high food intake, the high blood sugar level and the enormous sugar excretion indicate a very definite diabetes. The successful production of pancreatic diabetes in the rabbit opens a new field in the study of carbohydrate metabolism since hypophysectomy, hepatectomy, etc., are readily carried out in this excellent laboratory animal.

9552

Nutritional Dermatoses in Rats.

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Hitherto all scaly dermatoses produced in rats by means of diets have been broadly characterized as pellagra or pellagra-like. Failure properly and exactly to describe the scaly diseases of the rat and to differentiate one disease from another has led to confusion and controversy in the field of nutritional investigation. At least 3 scaly dermatoses can be produced in the rat and prevented or cured by nutritional means. They are due to (1) vitamin H deficiency (egg-white injury), (2) vitamin B_6 deficiency, and (3) lactoflavin deficiency.

(1) Vitamin H[1] deficiency disease is the name given to the general disorder experimentally produced in the rat by inclusion of a high proportion (15 to 40%) of egg-white in an otherwise well-balanced diet which contains all the well-known vitamins. Administration of vitamin H cures this condition. Rats kept on a diet of this kind exhibit in from 4 to 6 weeks typical symptoms of seborrheid[2] dermatitis, such as erythema, intertrigo particularly around the neck and genitalia, a brown crusting chiefly over the back that is similar to "cradle cap," and scaling that progresses from the areas of intertrigo to involve the entire surface of the body and that leads finally to a generalized exfoliative dermatitis. Generalized alopecia and exquisite pruritus are also manifest. The hind legs and the ears escape involvement. Excoriations heal slowly. Skin abscesses are rare; when present they are ecthyma-like. Mild sublingual ulcers are fairly common.

The microscopic picture reveals that in the earliest stages of the disease there is edema in the upper portion of the corium; there is also acanthosis with intercellular and intracellular edema. *Altération cavitaire* and spongiosis of the stratum spinosum precede the formation of intra-epithelial vesicles. There is a questionably increased

1 Boas, M. A., *Biochem. J.*, 1927, **21**, 712; Parsons, H. T., *J. Biol. Chem.*, 1931, **90**, 351; György, P., *Z. f. ärztl. Fortbild.*, 1931, **28**, 377; also, *Handbuch der Kinderheilkunde* (Pfaundler and Schlossmann), Berlin, F. C. W. Vogel, 1935, **10**, 58.

2 Moro, E., *Ekzema infantum und Dermatitis seborrhoides*, Berlin, J. Springer, 1932, p. 4.

activity of the sebaceous glands. Surprisingly little cellular infiltration accompanies the marked changes in the epidermis and the edema in the corium. The fixed connective tissue cells are increased. The connective tissue is slightly edematous and dissociated. Marked hyperkeratosis and parakeratosis are next observed, and most striking is the presence of numerous dilated and hyperemic blood vessels throughout the corium. There is no evidence of endovasculitis and no perivascular infiltration. As the skin lesions undergo involution the normal epithelium is restored with slight damage to the fibrous connective tissue but with no disturbance of the elastic fibers.

(2) Vitamin B_6[3] deficiency in rats produces the disease entity hitherto called "rat pellagra" or, better, "rat acrodynia." Rats kept on a diet devoid of the vitamin B complex and supplemented with vitamin B_1 and lactoflavin manifest in from 6 to 15 weeks symmetrical dermatitis of the peripheral parts of the body, the paws, nose, mouth and ears. There is no pruritus. Scaliness and edema are evident. Alopecia usually does not occur; at most it is slight. Abscesses form particularly around the mouth and cheeks; sublingual necrotic ulcers are not uncommon. In the advanced stages scaliness over the trunk is seen. Slight or no loss of hair accompanies the scale formation.

Microscopically hyperkeratosis is observed, but not the extreme parakeratosis of the vitamin H deficiency. The epidermis here and there displays acanthosis and spongiosis, but true intra-epithelial vesiculation is not seen. The corium differs from that seen in the disease due to vitamin H deficiency in that there is a diffuse though not marked infiltration of exudative cells. Some lymphocytes are present and an occasional leucocyte, in addition to the increased amount of connective tissue cells and the edema. A few hyperemic vessels are seen here and there but they are by no means as abundant as in the disease due to vitamin H deficiency. Secondary infection is observed, with abscesses in which there is a focal necrosis. Large numbers of polymorphonuclear leucocytes are seen in the corium as well as in the follicles.

(3) Lactoflavin[3] deficiency is produced by placing rats on an experimental diet from which only the lactoflavin component of the vitamin B_2 complex has been excluded. After 8 to 15 weeks the rats show mild but definite dandruff-like flakes or scales without or with more or less symmetrical loss of hair.

Hyperkeratosis of mild degree is observed microscopically. The

[3] György, P., *Biochem. J.*, 1935, **29**, 741.

epidermis is not particularly altered; it is perhaps slightly thickened. In the upper corium there is occasionally very slight exudation. As the disease advances, the number of follicles diminishes, and there appears to be some slight increase in the activity of the sebaceous glands.

The production by nutritional means of 3 scaly dermatoses in rats, each with a different cause, indicates that the factors of nutrition and metabolic disturbance play an important etiologic rôle in similar conditions in man.

9553

Relative Toxicities and Therapeutic Values of Three Chemotherapeutic Agents of the Sulphonamide Type.

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Since the original report of Domagk[1] on the protective effects of 4'-sulfonamido-phenyl azo-7-acetylamino-l-oxynaphthalene-3, 6-disulfonate of sodium (prontosil) in mice infected with *Streptococcus hemolyticus,* an extensive experimental and clinical literature[2–11] has developed on the use of this and related compounds in streptococcal and other types of infections. Nevertheless, information on the relative toxicity of these several products is either incomplete or relatively limited. Further, their therapeutic efficiencies have been only imperfectly evaluated due to lack of extensive comparative data on the therapy of bacterial infections of standard virulence.

Toxicity. The oral lethal dosages of prontosil and 4-(4'-aminobenzol-sulfonamide)-benzol-sulfonamide (Disulon) cannot be accurately established due to high tolerance and the limited gastric

1 Domagk, G., *Angew. Chemie,* 1935, **48**, 657.

2 Levaditi, C., and Vaismon, A., *Presse med.,* 1935, **103**, 2097.

3 Levaditi, C., and Vaismon, A., *Compt. rend. Soc. de biol.,* 1936, **121**, 803.

4 Colebrook, L., and Kenny, M., *Lancet,* 1936, **1**, 1279.

5 Buttle, G. A. H., Stephenson, D., Smith, S., and Foster, G. E., *Lancet,* 1937, **1**, 1331.

6 Gray, W. H., Buttle, G. A. H., and Stephenson, D., *Biochem. J.,* 1937, **31**, 724.

7 Long, P. H., and Bliss, E. A., *J. A. M. A.,* 1937, **108**, 32.

8 Rosenthal, S. M., *Pub. Health Rep.,* 1937, **52**, 48.

9 Proom, H., *Lancet,* 1937, **232**, 16.

10 Rosenthal, S. M., *Proc. J. Pharm. and Exp. Therap.,* 1937, **60**, 117.

11 Halpern, B. N., and Mayer, R. L., *Presse Med.,* 1937, **40**, 747.

capacities of the animals. However, the M.L.D.'s of these 2 compounds by this route exceed 40 gm. per kilo of body weight, respectively.

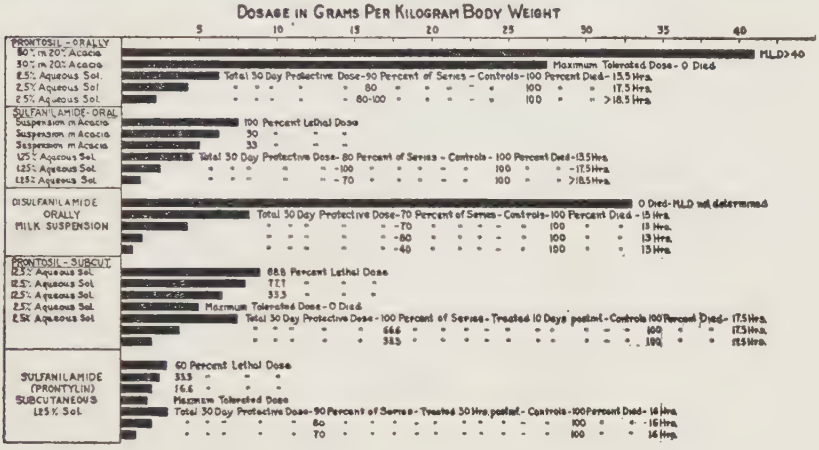
In an acacia-suspension the 50% oral M.L.D. dosage of *p*-amino benzene sulphonamide (prontylin or sulphanilamide) for mice is 6.25 gm. per kilo. Large dosages of this agent can be administered only in the form of a suspension, because of its low aqueous solubility.

For albino mice the M.L.D. of prontosil administered subcutaneously in an isotonic aqueous menstruum lies between 6 and 8 gm. per kilo. The corresponding dosages of prontylin in water, 20% acacia suspension, and olive oil[8] are 2.75, 3.75 and 4.0 gm. per kilo body weight, respectively. The subcutaneous toxicity of disulon, because of its low aqueous solubility, was not determined. Solutions of soluble (sodium or hydrochloride) salts of prontylin are strongly irritant. Reliable toxicological data with such solutions are not obtainable on parenteral administration.

Effect on Growth-rates. The growth-rates of young (50 gm.) rats (maintained on a complete diet) following the subcutaneous administration of prontosil (as a 40% aqueous suspension) in dosages of 2.0 gm. per kilo (16.6% of a single M.L.D.) per day lagged 6.3% behind that of the controls after a 9-week period. The oral administration of 10 gm. per kilo (27% of a single M.L.D.) per day from the 10th to the 12th week inclusive resulted in a 13% lag. This effect was purely a disturbance of alimentation, however, in that the growth-rate of this group 2 weeks after discontinuance of medication exceeded that of the control series by 8.7%. Oral daily dosages of 0.25 gm. of prontylin per kilo (3.3% of a single M.L.D.) over a 9-week period were without influence on the growth rates. Increasing the dosage to 0.75 gm. per kilo (10% of a single M.L.D.) from the 10th to the 12 weeks did not influence the normal growth-curves. P-acetyl-amino-benzene-sulfonamide, the primary conjugation-product of prontylin, in the organism, differed in no respect from prontylin under similar conditions in its effect on the growth-rate.

Therapeutic Efficiency. In our experiments we consider the minimal protective dose to be the total dose, administered over a 10-day period, which protects for 30 days 70 to 100% of animals infected intraäbdominally with 0.3 cc. of a 1:100 saline suspension (800,000 to 1,200,000 bacteria per cc.) of our C-391-2 culture of *β-Streptococcus hemolyticus*. This strain of streptococcus of human origin was obtained at necropsy and subsequently isolated in pure culture.

A high mouse-virulence was maintained by successive passages through mice. Treatment was begun 1 to 2 hours after infection and repeated at 6-hour intervals for the first 24 hours and daily thereafter up to and including the 10th post-infection day.



The figure shows that the minimal protective dose of each product has the following order: prontosil > prontylin > disulon. Moreover, the minimal protective dose varies directly with the virulence of the culture. This point amply confirms the statements of Long and Bliss[7] that effective treatment of experimental streptococcal infections with these chemotherapeutic agents must be predicated on a survival period of reasonable length after institution of therapy (13 or more hours in our experience with mice).

Summary and Conclusions. The anti-streptococcal effects of prontosil on oral administration are somewhat superior to those noted after subcutaneous injection. This confirms Rosenthal.[8]

The chemotherapeutic efficiency of prontylin by the subcutaneous route is superior to that noted after oral medication. This confirms Proom[9] and Rosenthal.[8] The observed differences in efficiency by these 2 routes of administration were not due to the greater volume of fluid administered by the subcutaneous route, in that normal saline injections alone were of no therapeutic value. However, the superiority of the parenteral route from a therapeutic standpoint is open to question, when due consideration is given to the low solubility of the compound and the close similarity between the time intervals, at which the peak blood concentration occurs, after medi-

cation with equal dosages of prontylin by these 2 routes. (Marshall, *et al.*[12])

Prontylin orally appears to be 1.8 times more effective than prontosil for low-grade infections and 1.4 times more effective for high-grade infections. The therapeutic margin of safety of prontosil administered orally, however, is quite superior to that of prontylin.

Disulon orally in dosages of 40 gm. per kilo does not produce symptoms referable to the central nervous system. This compound, due to its lower toxicity, better tolerance and the greater protective efficiency of unit dosages in the presence of infections, has a therapeutic margin of safety quite superior to that of prontylin. The observed superior therapeutic efficiency of disulon as compared with prontylin confirms similar observations of Rosenthal.[10]

9554

Sodium Chloride Content of Gastro-Intestinal Secretions.*

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The necessity for replacing the sodium chloride carried away when excessive amounts of gastro-intestinal secretions are lost, as by vomiting, or drainage from biliary or intestinal fistulæ, has been repeatedly emphasized. In fact, the value of sodium chloride solutions in such instances is so well known that a definite tendency exists for their use in all patients requiring parenteral fluids. This procedure is not without risk, since the development of edema from the administration of excessive amounts of sodium chloride to sick patients is not uncommon. To avoid this mistake and at the same time provide sufficient salt, the physiologically and chemically minded surgeon knows about the metabolism of these electrolytes and fits his treatment to the needs of the individual patient.

The purpose of this paper is to show the amount of sodium chloride present in various gastro-intestinal secretions obtained from

[12] Marshall, E. K., Jr., Emerson, K., and Cutting, W. C., *J. A. M. A.*, 1937, **108**, 953.

* This investigation was aided by a grant from the Horace H. Rackham School of Graduate Studies.

surgical patients and, in particular, to draw attention to these amounts in relation to the quantity of sodium chloride in physiological saline or Ringer's solution, the common materials used for sodium chloride replacement.

Sodium was determined by the Butler-Tuthill method.[1] Estimations of chloride were made by the Wilson and Ball modification[2] of Van Slyke's method, except for a few of the first determinations done on thin vomitus, in which the Volhard-Arnold principle was followed. All analyses were run on samples from 24-hour collections.

The data are shown in Table I. The vomitus was collected from both preoperative and postoperative patients; the bile came from common duct T-tubes; the enterostomy drainage was from the mid-

TABLE I.
The Sodium Chloride Concentration of Gastro-intestinal Secretions and of Physiological Saline and Ringer's Solution.

Material	Patient No.	Sodium		Chlorine		Sodium Chloride
		Gm./L.	m.Eq./L.	Gm./L.	m.Eq./L.	Gm./L.
Vomitus	1			0.73	20	1.20
	2			2.60	73	4.30
	3			2.85	80	4.72
	4			3.33	94	5.50
	5			2.15	60	3.55
	6			1.21	34	2.00
	7			3.40	96	5.60
	8			1.00	28	1.65
	9			1.09	31	1.80
	10			1.27	36	2.10
	11	0.38	16	0.73	20	1.20
	12	0.54	23	3.78	106	6.24
	13	0.80	35	2.00	56	3.30
	14	0.07	3	1.68	47	2.78
	15	0.42	18	1.16	33	1.92
	16	0.48	21	0.91	26	1.50
	17	1.79	78	3.11	88	5.12
	18	0.97	42	3.31	93	5.46
Hepatic Bile	19	3.26	142	3.69	104	6.09
	20	3.30	143	3.83	108	6.31
	21	3.39	147	4.62	130	7.62
	22	3.54	154	4.32	121	7.13
Enterostomy Drainage	23	2.44	106	2.62	74	4.32
	24	2.19	95	3.65	103	6.02
Phys. Saline		3.34	145	5.15	145	8.50
Ringer's Solution	Na	3.34	145	5.15	145	8.50
	K			0.14	4	0.30
	Ca			0.13	4	0.20

1 Butler, A. M., and Tuthill, E., *J. Biol. Chem.*, 1931, **93**, 171.
2 Wilson, D. W., and Ball, E. G., *J. Biol. Chem.*, 1928, **79**, 221.

ileum of 2 patients with intestinal obstruction. The figures for sodium chloride were calculated from the chloride determinations as if all the chlorine were present as sodium chloride.

The point we wish to emphasize is that excepting 2 instances (sodium in patients 21 and 22) the concentration of sodium chloride in the gastro-intestinal secretions examined was always less than the concentration of sodium chloride in physiological saline or Ringer's solution.

This suggests for patients losing gastro-intestinal secretions and requiring fluids parenterally that the sodium chloride lost may be replaced by giving a volume of physiological saline or Ringer's solution equal to the volume of gastrointestinal secretion lost. The possibility of preventing a deficiency of sodium chloride by this volume for volume rule is being investigated now in a series of surgical patients.

9555

Toxicity of meta-Substituted Phenols to *Paramecium caudatum.*

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The duration of life of individual paramecia was measured at various concentrations of antiseptic agents, at 25 ± 1°C., and at pH 7.6. Duplicate runs were made on separate pure cultures of *Paramecium caudatum,* using an average of 20 organisms at each concentration in each run. The solution of the toxic agent and the 0.5% oat straw infusion paramecium culture were measured from microburettes, mixed in small depression slides, and placed in a stage thermostat for microscopic observation. Death was considered to occur when motility ceased. Morphological alterations indicated that the organisms actually were dead, especially the formation of clear spherical blisters about the periphery, the disappearance of the vacuoles, and a darkening and clumping together of the cytoplasmic elements. The death times at each concentration in each run were averaged. The average of the probable error in the death times was 3.3%.

The results of each run on each compound were plotted separately, log c against log t. The straight lines obtained conform to the

Ostwald[1] toxicity equation, $c^n t = k$, where c is the concentration of toxic agent in mols per liter, t is the death time in seconds, and n and k are constants. For each run, n was determined as the slope, log t divided by log c, of the balanced line drawn through the points. The 2 values of n obtained for each compound were averaged and the probable error in n was expressed statistically, averaging 2.8%. From the average value of n for a given compound, k was calculated by substitution in the equation for all the points in both runs, and the probable error in the average k was expressed statistically, averaging 3.6%. From the average values of n and k, the straight lines in Fig. 1 and 2 were drawn. The points are the average of the death times in the 2 runs.

The numerical values of the constants, n and k, are given in Table I. The 2 constants may be combined into a single constant, the nth

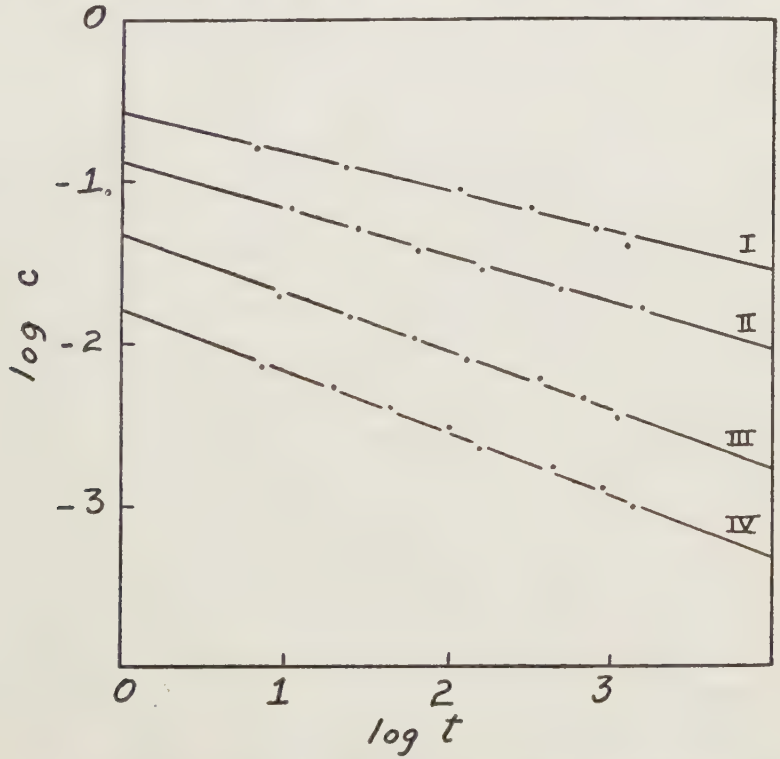

FIG. 1.

Kinetics of death of *Paramecium caudatum* under the influence of: I. m-amino-phenol; II. Resorcinol; III. Resorcinol monomethyl ether; IV. m-nitro-phenol.

1 Ostwald, Wo., *Arch. ges. Physiol. (Pfluger's)*, 1908, **120**, 19.

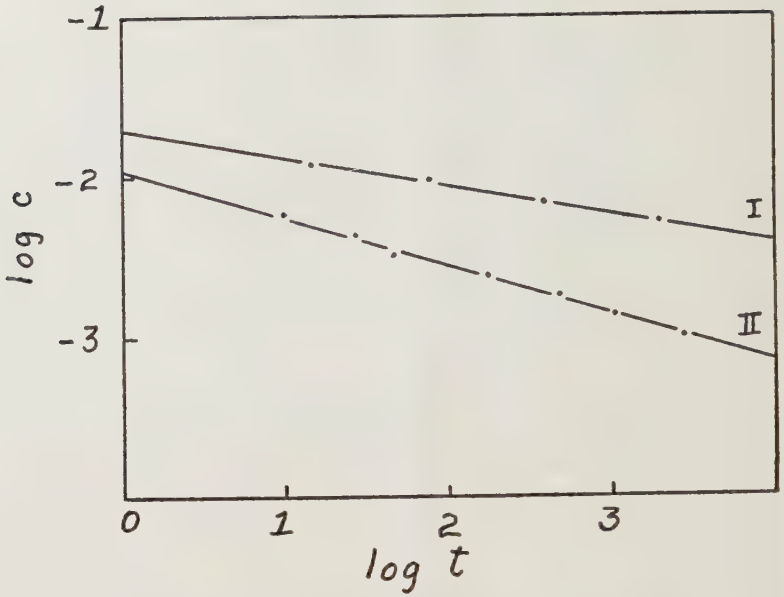

FIG. 2.
Kinetics of death of *Paramecium caudatum* under the influence of: I. m-cresol; II. m-chloro-phenol.

root of k, which is the concentration of toxic agent required to bring about death in unit time, one second. The nth root of k might be called the *Toxicity Coefficient*. Since surface tension might be considered the cause of death, the surface tension, of the highest concentration of each toxic agent used, was measured by the Du Nuoy method. The surface tension, in all cases, was within the range of surface tension that was normal for the oat straw infusion cultures used, 62.8 to 72.1 dynes per cm.

Inspection of Table I shows a relatively wide range of variation

TABLE I.
The Constants, n and k, in the Ostwald Toxicity Equation.

Compound	n	k	Toxicity Coefficient $\sqrt[n]{k}$	Surface Tension of strongest soln. dynes/cm.
m-chloro-phenol	3.37 ± .02	2.50 ± .11 × 10–7	.0109	72.5
m-nitro-phenol	2.63 ± .3	2.01 ± .05 × 10–5	.0164	72.8
m-cresol	5.71 ± .02	1.80 ± .02 × 10–10	.0196	64.5
Resorcinol mono-methyl ether	2.73 ± .07	2.44 ± .09 × 10–4	.0475	65.1
Resorcinol	3.42 ± .03	9.99 ± .23 × 10–4	.1328	69.2
m-amino-phenol	4.15 ± .03	3.99 ± .30 × 10–3	.2646	65.2

in the constants n and k for this closely related group of compounds. With the constituents arranged in increasing order of the nth root of k or decreasing antiseptic efficiency, as above, it is interesting to note that Labes and Jansen[2] arranged the substituents in phenol in the same order of decreasing ability to coagulate serum albumin, chloro, nitro, methyl, hydroxyl, indicating a definite parallelism between antiseptic efficiency and ability to coagulate proteins. This parallelism gives supporting evidence to Bancroft's[3] colloidal coagulation theory of disinfectant action. An analogy can be shown between the Ostwald toxicity equation and the Ishizaka[4]-Gann[5] equation for the kinetics of the coagulation of colloidal $Al(OH)_3$ by KCl

$$t = \frac{2.303}{ac^n(1 + b)} \log \frac{b + x}{b(1 - x)}$$

where x is the fraction coagulated in time t when the concentration of KCl is c, the terms a, b, and n being constants. If the time to a given degree of coagulation, t_s, is taken, this equation reduces to $c^n t_s = k$, identical, in form and range of magnitude of n, with the Ostwald toxicity equation. The Ostwald toxicity equation can therefore be considered to measure the time until a sufficient degree of protoplasmic coagulation has occurred to produce death.

Conclusion. Additional evidence is presented to support the Bancroft colloidal coagulation theory of disinfectant action. A new measure of the efficiency of antiseptic agents, the Toxicity Coefficient, is defined on the basis of the Ostwald toxicity equation.

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2 Labes, R., and Jansen, E., *Arch. exp. Path. Pharm.*, 1930, **158**, 1.

3 Bancroft, W. D., and Richter, G. H., *J. Phys. Chem.*, 1931, **35**, 511.

4 Ishizaka, N., *Z. physik. Chem.*, 1913, **83**, 97.

5 Gann, J. A., *Kolloidchem. Beih.*, 1916, **8**, 63.

9556 P

An Agent, Transmissible to Mice, Obtained during a Study of *Pemphigus vulgaris.*

ARTHUR W. GRACE AND FLORENCE H. SUSKIND. (Introduced by E. F. DuBois.)

From the New York Hospital and Department of Medicine, Cornell University Medical College, and from the Skin and Cancer Unit, New York Post-Graduate Medical School and Hospital, Columbia University.

The frequent symmetrical distribution of the cutaneous manifestations of *Pemphigus vulgaris* and their resemblance to trophic lesions as well as the limitation of the pathologic changes to the skin and nervous system have suggested a neurotropic etiologic agent of the disease. Carol[1] has summarized the work of earlier investigators in this field and has recorded his own results following intracerebral inoculation of pemphigus material into rabbits, guinea pigs, monkeys, and mice. He concluded that a specific virus of pemphigus could not be demonstrated by the methods hitherto employed. We, however, felt that there was more likelihood of the transmission of an etiologic agent of pemphigus to animals if the resistance of the animals was previously lowered by irradiation. The results obtained by the inoculation of blister fluid and spinal fluid from 3 cases of *Pemphigus vulgaris* into the brains of irradiated mice are recorded here.

Case I, D. R., an adult female, had been sick for 6 months when bacteriologically sterile blister fluid was obtained 23 days before death. Five mice which received a generalized x-ray dose of 400 r. were inoculated intracerebrally with 0.03 cc. of fluid. Three weeks later all were sick. One was killed on the 22nd day, another died on the 94th day, and the remainder recovered. A 40% bacteriologically sterile emulsion in physiologic saline of the brain of the killed animal was used for passage. All of the animals which received the brain emulsion became sick in the second week after inoculation and died by the 27th day. The active agent has since been transmitted for 46 passages and at present 0.03 cc. of a 5% emulsion of bacteriologically sterile infectious brain material kills 90 to 100% of the mice in 7 days. The animals usually show signs of illness 2 to 4 days after inoculation. The coat becomes rough, the back humped, and they appear to walk on the toes. Loss of weight and pallor often become apparent at this time. Later the humping of the

[1] Carol, W. L. L., Prakken, J. R., Ruiter, M., Snidjers, E. P., and Wielenga, D. K., *Arch. f. Dermat. u. Syph.*, 1937, **175**, 265.

back increases markedly, ataxia may develop, and wasting and apathy progress until the animal dies. Hyperirritability frequently occurs and is sometimes so pronounced that the slightest tactile stimulus causes the animal to leap high in the air. Occasionally a hyperirritable animal shows rapid, apparently uncontrollable, head movements.

Histologic examination has as yet been confined to the brain and meninges which show necrotic areas filled with polymorphonuclear leucocytes, perivascular collections of mononuclear cells and occasional plasma cells. All of the brains examined histologically in this study were bacteriologically sterile on aerobic and anaerobic culture.

It has also been found possible to infect non-irradiated mice by intracerebral inoculation of bacteriologically sterile infectious brain material from irradiated mice and vice versa. The active agent was transmitted from non-irradiated to non-irradiated mice for two passages. No more than 2 such passages were made as it was felt that the active agent could not be maintained as well thus as in irradiated animals. The mortality rate in these 2 passages was very similar to that observed when non-irradiated mice were inoculated with infectious irradiated brain, namely, 50-60%.

The clinical and histologic pictures in the infected non-irradiated animals were identical with those observed in the infected irradiated mice.

Case II, W. H., an adult male, had been sick for one year when bacteriologically sterile spinal fluid was obtained in December, 1936. He was alive in July, 1937. The fluid was inoculated intracerebrally into 5 irradiated mice. Passage was made from a sick animal killed on the 34th day, using a 40% emulsion of bacteriologically sterile infectious brain material. Although the active agent increased in virulence with successive passage so that, later, a 20% bacteriologically sterile emulsion could be employed it could not be transmitted for more than 7 passages. Clinical and histologic pictures of the infected animals were identical with those found in Case I.

Case III, P. R., an adult male, was ambulatory and had been sick for 3 weeks when bacteriologically sterile blister fluid was obtained. He was not seen again. The fluid was inoculated intracerebrally into 7 irradiated mice. Six showed definite signs of illness in 10 days and one died on the 3rd day, a second was killed on the 14th day, and another died on the 30th day. An unsuccessful attempt at passage was made with the brain of the animal which died 3 days after inoculation. Histologic examination of the brain of the animal

which was killed 14 days after inoculation showed perivascular collections of mononuclear cells in the meninges.

Guinea pigs were not susceptible to the strain obtained from Case I inoculated either intracerebrally or subcutaneously.

The activity of infectious brain material could be preserved for at least 5 days in the ice box at 8°C.

Control animals were of 3 groups. Both control and test animals were irradiated at the same time. One group remained uninoculated and was observed for the effects of irradiation. A second group was inoculated intracerebrally with 0.03 cc. of bacteriologically sterile blister fluid obtained from a normal human subject by the application of cantharides plaster. The third group was inoculated intracerebrally with 0.03 cc. of a 40% emulsion of bacteriologically sterile irradiated normal mouse brain. Passages were made of the brains of the control mice of the latter 2 groups at the same time interval and in the same concentration as the infected animals. Forty to 50% of the control animals of each group died. Control mice died usually without any apparent period of illness, and histologic examinations of their brains did not show the inflammatory changes observed in the infected animals.

Summary. Definite clinical and pathologic changes were produced in irradiated mice inoculated intracerebrally with bacteriologically sterile blister fluid from 2 cases and spinal fluid from one case of *Pemphigus vulgaris*. In 2 of these cases serial transmission of the active agent was carried out with bacteriologically sterile material. Identical changes were produced in a smaller proportion of non-irradiated mice using infectious irradiated brain material as the primary inoculum. Control animals did not show the clinical and pathologic changes of the infected animals. Further study is necessary to determine the nature and properties of the active agent and its relation to *Pemphigus vulgaris*.

9557 P

Physicochemical Changes in Blood Serum of Patients with Schizophrenia Treated by Hypoglycemic Shock.

José Zozaya.

From Gladwyne Research Laboratory, Gladwyne, Pa.

Since the report of Sakel[1] on the successful treatment of certain cases of schizophrenia by producing a hypoglycemic shock with the use of insulin, there has been increasing interest in the study of the physiological, psychological and chemical changes that occur during the treatment.

Our previous studies on mental and nervous diseases have been concerned mainly with the physicochemical changes that occur in the blood serum during the different stages of acute insanities. With this previous experience we thought it of interest to study the changes that may occur during the shock treatment with insulin.

The method of treatment is, with slight modifications, essentially that suggested by Sakel.[2]

In the present communication we shall not be concerned with the clinical results or changes in the individuals treated, but exclusively with the changes observed at different periods of the treatment. This report includes the result of 23 different days of treatment of 8 patients. The bloods were taken during fasting (*i. e.*, before the insulin was given), during the coma or deep shock, and finally 15 to 20 minutes after the administration of glucose intravenously, given to terminate the shock or coma. At the same time that blood was taken for the study of the proteins, a sample of oxalated blood was also taken to determine the amount of glucose.

The methods used in this study are the same as those reported previously.[3]

We notice a change in the specific gravity at the time of the coma, which continues after termination. This increase we attribute to dehydration, which is probably due to the marked perspiration before the patient gets into shock. This loss of water can easily account for this change in the specific gravity. The changes in specific gravity at coma varied from a decrease of .0002 to an increase of .0026.

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1 Sakel, M., *Wien. med. Wchnschr.*, 1934-35, **84-85**, 1211.

2 Sakel, M., *Neue Behandlungs methode de Schizophrenie Verlag Moorits Perles*, Vienna, 1935.

3 Zozaya, J., *J. Biol. Chem.*, 1935, **110**, 599.

The changes in viscosity are not marked in the average figures but in individual cases there were times in which we observed a marked increase up to 0.15.

We notice a marked increase in the total protein at the coma, with a further increase at termination. This increase in part at least we attribute to the concentration of the blood by dehydration, but there is no doubt a further mechanism which actually increases the amount of total protein, for there is a certain shift in the proportions of the different protein fractions (percentage of total protein, of albumin, of pseudoglobulin, and of euglobulin). This increase becomes most marked after termination, at which time we observe in the great majority of cases a marked increase in the euglobulin fraction.

Of the 23 bloods examined, 17 showed a definite increase in euglobulin, or 74%. Of those which did not show the increase, 3 came from the same patient who never reacted favorably to the treatment.

This increase in the euglobulin we feel is the most significant of the changes observed, for it occurs immediately after the injection of glucose, the average increase being 0.39 gm., a significant amount.

With this change we observe the corresponding changes in "bound water" and "free water", the latter decreasing 2.6 cc. per 100 cc. of blood from the fasting time.

The albumin-globulin ratio can be seen to have changed from 1.86 during fasting to 1.57 after termination, with a slight decrease during coma.

The glucose percentage in the blood is interesting, for we observed as low as 9 to 48 mg. per 100 cc. of blood during coma. It is of interest to note that different individuals have different sugar levels during coma.

The changes in the cholesterol in the serum are slight and we do not give them any importance.

9558 P

Treatment of Alcoholic Cirrhosis of the Liver with High Vitamin Therapy.

ARTHUR J. PATEK, JR. (Introduced by W. W. Palmer.)

From the Research Division of Chronic Diseases, Department of Hospitals, City of New York, and the Department of Medicine, College of Physicians and Surgeons, Columbia University.

The recent studies of polyneuritis,[1, 2, 3] pellagra,[4] and beriberi heart[5] have indicated that these diseases are due to nutritional lack, whether or not they are associated with alcoholism, and that they are alleviated by diets and medication rich in the vitamin B complex. It seemed pertinent to make a similar investigation of patients with alcoholic cirrhosis of the liver, to inquire into their diets, and to study the effect of high vitamin therapy on the course of this disease.

Thirteen patients with the diagnosis of alcoholic cirrhosis were observed. In all instances they were heavy, chronic drinkers of alcohol. Symptoms of gastro-intestinal disorder dominated the histories of these patients preceding their illness. In most instances diet and digestive functions were abnormal. In 9 cases the caloric intake was very low. Meat, green vegetables and fruit seldom were eaten. Corresponding weight losses occurred, varying from 9 to 49% of the usual weight in 10 of 13 cases.

Clinical Data. Although the diagnosis of cirrhosis cannot be proved without biopsy, the evidence of chronic liver injury seemed adequate. The diagnosis was based usually upon the history of alcoholism and the presence of a hard, palpable liver, esophageal varices, ascites, reduced dye excretion test, positive Takata Ara test, and altered serum proteins. All these signs were not exhibited by all cases.

In 10 cases the liver was palpably enlarged. Nine patients had ascites, and of these 6 also had edema of the ankles.

There was likewise evidence of specific malnutrition, notably of the vitamin B complex. Peripheral neuritis, glossitis, gastric anacidity, pellagrous dermatitis, persistent tachycardia, anemia, and purpura were common.

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1 Wechsler, J. S., *Arch. Neur. and Psychiat.*, 1933, **29**, 813.

2 Minot, G. R., Strauss, M. B., and Cobb, S., *New Eng. J. Med.*, 1933, **208**, 1244.

3 Jollife, N., Colbert, C. N., and Joffe, P. M., *Am. J. Med. Sci.*, 1936, **191**, 515.

4 Spies, T. D., Chinn, A. B., McLester, J. B., *J. A. M. A.*, 1937, **108**, 853.

5 Weiss, S., and Wilkins, R. W., *Trans. Assoc. Am. Phys.*, 1936, **51**, 341.

Observations have been made during a period of from 3 to 13 months. The patients were given diets which contained CHO 305, Protein 102, Fat 120. The standard daily medication contained Oleum Percomorphum 30 mins., orange juice 12 oz., Valentine's Liver Extract 2 oz., Vegex 3 drams, and parenteral crystalline vitamin B_1.* The latter was injected in daily doses varying between 4 and 10 mg. In 2 instances vitamin B_1 alone was administered.

Three patients died and 10 have been making steady improvement. General clinical improvement was evidenced by increased appetite, gain in weight, return of strength. In 7 cases a measured reduction in liver size occurred. Partial or complete recovery from associated conditions, such as glossitis, dermatitis, anemia, and neuritis generally was noted in those who improved.

There have been striking changes in several patients with ascites. Three patients previously had experienced repeated paracentesis; a fourth patient had received one paracentesis before entry, and 2 others with signs of ascites did not need tapping. In this group, after varying intervals of time there occurred diuresis and loss of ascites without recurrence.

Laboratory data reflected corresponding improvement: in 11 cases a rise of serum albumin; in 5 cases improved bromsulphalein test; in 6 cases reversal of Takata Ara test to negative. Whereas "spontaneous" improvement of mild cases is not uncommon, such changes as these in decompensated cirrhosis of the liver appear to be outside chance expectations.

Conclusions. 1. There appears to be a significant relationship between nutritional deficiency and alcoholic cirrhosis of the liver. 2. It is believed that patients with alcoholic cirrhosis of the liver are benefitted by high vitamin therapy.

* The crystalline vitamin B_1 was supplied by the courtesy of Merck and Co., Rahway, N. J., and of the Winthrop Chemical Co., New York City.

9559 P

Potentiating Influence of Urine on Sulfanilamide's Bacteriostatic Effect on *E. coli in vitro.*

Ralph R. Mellon and Lawrance E. Shinn.

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In a previous publication[1] one of us (Mellon) reported a potentiative effect of sulfanilamide on hemolytic streptococci when the organisms had been exposed to physiological salt solution during the process of dilution preliminary to seeding the test cultures. If this exposure was omitted and dilution in broth substituted, the remarkable bacteriostatic effect of sulfanilamide in low concentrations was not noted. In the presence of normal human serum the effect is enhanced. In other words, a sterilizing effect is obtained from the combined action of these minimal factors which exceeds many times a simple summation effect.

Kenny, Johnston, and von Haebler[2] have recently reported a very favorable series of clinical cases of *E. coli* infection of the urinary tract which were successfully treated with sulfanilamide. They showed that with oral medication of 1.5 gm. a day for 5-7 days sterilization of the urine was obtained during the period of treatment in all of 46 cases of infection with a typical *E. coli.* The concentration of free sulfanilamide obtained in the urine of treated cases ranged from less than 1:100,000 to 1:1,000. It was shown experimentally that the static or bactericidal action of the compound *in vitro* was roughly proportional to its concentration.

It appeared to us that a potentiation similar to that displayed by saline for hemolytic streptococci might be anticipated in this instance with the culture medium itself (the urine) playing the rôle of the potentiating agent. Bacteriostatic tests were accordingly made on a strain of *E. coli* freshly isolated from a case of cystitis which had had no sulfanilamide therapy. Normal pooled urine was adjusted to pH 7.2 and sterilized by Seitz filtration. The broth employed in the following experiments was a 2% proteose-peptone beef infusion of the same pH. Cultures were grown either in urine or broth for 18 hours, and then serially diluted in either urine or broth to the concentration required for seeding the test cultures which were of 2 cc. volume. All seedings and sulfanilamide-additions were in quantities of 0.1 cc. The test cultures were then incu-

1 Mellon, R. R., and Bambas, L. L., *Med. Record,* 1937, **246**, 247.

2 Kenny, M., Johnston, F. D., and von Haebler, T., *Lancet,* 1937, **233**, 119.

bated without agitation for 48 hours. Counts were made by plating 0.5 cc. from each at 0, 6, 24, and 48 hours.

TABLE I.

Effect of the Original Culture Medium, Diluent, and Test Medium on the Bacteriostasis of *E. coli in vitro*. Figures averaged from 4 determinations.

Culture medium	Diluent	Test medium	Conc. sulfanil.	Counts per cc. at 0 hr.	6 hr.	24 hr.	48 hr.
Broth	Broth	Broth	0	19	*	*	*
,,	,,	,,	1:10,000	15	*	*	*
,,	,,	Urine	0	10	*	*	*
,,	,,	,,	1:10,000	14	*	*	*
,,	Urine	Broth	0	22	*	*	*
,,	,,	,,	1:10,000	16	*	*	*
,,	,,	Urine	0	10	*	*	*
,,	,,	,,	1:10,000	15	150	11	4
Urine	Broth	Broth	0	26	*	*	*
,,	,,	,,	1:10,000	19	*	*	*
,,	,,	Urine	0	10	*	*	*
,,	,,	,,	1:10,000	17	*	*	*
,,	Urine	Broth	0	48	*	*	*
,,	,,	,,	1:10,000	53	*	*	*
,,	,,	Urine	0	42	*	*	*
,,	,,	,,	1:10,000	61	400	1	0

*Uncountable; greater than 20,000.

The comparative effects of broth and urine as initial media, diluents and test media are shown in Table I. Of the 8 possible combinations only 2 show stasis and bactericidal action and these 2 (broth-urine-urine and urine-urine-urine) show it with great clarity.

TABLE II.

Effect of Initial Count and Concentration of Sulfanilamide on the Bacteriostasis of *E. coli* When Grown in Urine, Diluted in Urine, and Tested in Urine. Figures averaged from 4 determinations.

Initial count per cc.	Conc. sulfanil.	Counts per cc. at 6 hr.	24 hr.	48 hr.
1000	0	*	*	*
1000	1:5,000	10,000	5,000	0
1000	1:10,000	10,000	5,000	200
200	0	*	*	*
200	1:5,000	650	11	3
200	1:10,000	900	†	†
200	1:50,000	*	*	*
100	0	*	*	*
100	1:1,000	400	2	0
100	1:10,000	600	0	90
100	1:50,000	*	*	*
50	0	*	*	*
50	1:5,000	450	4	0
50	1:10,000	500	10	0

*Uncountable; greater than 20,000.
†Erratic; some sterilized while others grew out heavily.

The only conclusion which seems possible from this result is that broth exerts either a protective effect on the organism or an inhibitory effect on the drug and that it must be removed by the dilution in urine.

Table II is an attempt to evaluate the minimal concentration of sulfanilamide and the maximal initial count compatible with stasis. It is apparent that initial counts as high at least as 1000 per cc. yield good bacteriostasis with a concentration of 1:10,000. There is furthermore a suggestion that the concentration of 1:10,000 is very near the critical value for a fairly wide range of initial counts, which is particularly interesting in view of the frequent appearance of this particular value *in vivo*.

Initial experiments employing a pH of 6.0 rather than 7.2 indicate that the action of sulfanilamide is considerably enhanced in the more alkaline range. A concentration of 1:1000 at pH 6.0 appears to be effective in somewhat the same degree as a concentration of 1:10,000 at pH 7.2. This agrees with the observations of Helmholz.[3]

Practically, these results effect a correlation between *in vitro* tests and *in vivo* clinical results where, according to Kenny, disparities existed with the technic employed by her.

9560

Sulfanilamide and the Macrophage Response to Hemolytic Streptococcal Peritonitis in Mice.

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Because of an apparent disparity between the results of Long and Bliss[1] and Mellon, Cooper, and Gross[2] concerning the rôle of phagocytosis in experimental hemolytic-streptococcal infections in mice under treatment with sulfanilamide, the following inquiry was undertaken. The disparity later came to center about the relative importance of the neutrophils and the clasmatocytes in phagocytosis. In our study we had suggested the importance of strain-differences to explain the original disparity; although it was realized that our experimental set-up was not designed to answer the questions involved. This paper purports to do that.

3 Helmholz, H. F., *J. A. M. A.*, 1937, **109**, 1039.

1 Long, P. H., and Bliss, E. A., *J. A. M. A.*, 1937, **108**, 32.

2 Mellon, R. R., Gross, P., and Cooper, F. B., *J. A. M. A.*, 1937, **108**, 1858.

Technic. A representative technical set-up with our Stoddard mucoid strain follows. Thirty mice were injected intraäbdominally with 0.5 cc. of a 1:50,000 dilution of a 12-hour veal-infusion broth culture of the highly virulent Stoddard mucoid strain at 9:45 A. M. Subcutaneous injections of 0.8 cc. of a one percent aqueous solution of sulfanilamide were given to 15 of the mice at the end of 10 hours when the organisms were present in smears of the exudate.

Withdrawal of the peritoneal exudate was made at regular intervals after inoculation, beginning at 3 hours, and repeated at 2- or 3-hour intervals, except during the overnight period—and continuing in this manner usually until the end of the third day. In both control and test groups, certain of the mice were killed at varying periods and smears were made of their spleens, for subsequent examination. Another series of experiments was run with the C-203 strain, used by Long and Bliss, in order to compare the results with our Stoddard strain.

Results with the Stoddard Strain. The first cells to appear in the inflammatory exudate were the polymorphonuclear leukocytes, at the 4-hour period. In 6 hours lymphocytes and monocytes began to appear and after 8 or 10 hours the ratio of neutrophils to monocytes was roughly 3:2. At this stage there was no phagocytosis to speak of, and the heavily encapsulated organisms were easily seen free in the fluid.

Fourteen hours after inoculation and 4 hours after the first treatment the cell-pictures in the treated and untreated animal began to diverge. In the treated animals the neutrophils became necrotic, the monocytic cells greatly increased, and although the number of free encapsulated organisms was fewer, phagocytosis nevertheless was rare.

After 24 to 36 hours the macrophages and monocytes completely dominated the cell-picture, and there was a moderate amount of phagocytosis by the macrophages. The neutrophils at this period were very necrotic, insignificant in number, and played no part in phagocytosis. At the 52-hour period no organisms were seen free in the fluid in the peritoneal cavity, the cells were very scanty, and they were mostly of the polyblastic and macrophagic type, with an occasional cell actively phagocytic.

Corresponding with the replacement of the neutrophils by the macrophages at the 24-hour period, there was a rapid disappearance of the organisms from the peritoneum. Cultures at 24 and 48 hours were negative. The negligible degree of phagocytosis seen in the splenic smears, and the moderate amount in the peritoneal cavity suggested yet an additional unknown factor in the disappearance of the microörganisms.

In the untreated mice there was little phagocytosis by either neutrophils or macrophages of the myriads of streptococci present. The ratio of the former cell to the latter was 3 or 4 to one, at any period observed; and all these mice died in 24 to 72 hours.

Results with the C-203 Mucoid Strain. Without going into unnecessary detail we may say that in general comparable series of experiments were run on this strain. The cellular response and the phagocytosis were notably different from those seen with the Stoddard mucoid strain, there being very much less divergence in the cell-picture at the end of 24 hours following treatment with this C-203 strain. The neutrophilic component of the exudate was decidedly more in evidence and the phagocytosis of the organisms at all stages was more conspicuous in this cell. The phagocytic picture at different stages of the disease was in general a confirmation of the observations of Long and Bliss with this strain.

A third strain isolated from a serious case of Ludwig's angina was avirulent for mice and was rapidly phagocyted by the neutrophils in large numbers in from 3 to 6 hours after inoculation.

It is thus clear that strain-differences, even apart from the virulence-factor *per se* are of much importance in advancing the time of the appearance of the macrophages at the site of inoculation; and when the strain is lacking in virulence they may appear scantily and tardily, due presumably to the lack of necessity for them.

The remarkable fact that within 8 to 12 hours after the administration of sulfanilamide these macrophages have practically replaced the neutrophils first suggested to us a specific mobilizing influence of the drug on this highly important defensive cell. And yet the undoubted bacteriostatic effect of the drug *in vivo* may apparently accomplish the same purpose indirectly, by suspending the microörganisms' elaboration of necrobiotic substances which presumably are negatively chemotactic for the cells.

This consideration together with the difference in response with the C-203 strain make the latter explanation decidedly more probable. The Stoddard Strain may have possessed special resistance to phagocytosis by the neutrophils, thus requiring the presence of the macrophages to dispose of these organisms. All of our work to date is opposed to the conception of a direct and exclusive action of the drug on the organisms, as stressed originally by Long and Bliss,[1] but recently regarded by Long as untenable.[3] On the other hand, it appears as one mediated (and potentiated) by the host's tissues.[4]

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3 Long, P. H., personal communication.

4 Mellon, R. R., and Bambas, L. L., *M. Rec.*, 1937, **146**, 247.

Summary. Strain-differences in Group A hemolytic streptococci evoke marked differences in the inflammatory cell-response in mice undergoing treatment of experimental peritonitis with sulfanilamide. Some mucoid strains are readily phagocyted by the neutrophils; others seem to require the presence of macrophagic cells almost to exclusion in order to dispose of them. Some non-virulent strains, even in the absence of treatment, are phagocyted and destroyed by the neutrophils alone. Phagocytosis of virulent strains is conditioned by the previous bacteriostatic action of the drug, which appears as an indirect one.

9561

A Comparison of the Effectiveness of Alpha and Beta Lactose in the Control of Intestinal Reaction.*

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The reaction of the intestinal contents and its control has assumed increasing importance during recent years with the growing realization that acidity and alkalinity are important factors in the digestion and absorption of food and in the progress of various pathological conditions of the intestinal tract. With this knowledge has come an increased interest in the factors influencing the reaction and the methods for their control. One of us[1] has published data indicating that there is a certain constancy about the reactions of the various sections of the intestine which have the appearance of physiological constants and which the body strives to maintain. Unpublished results have confirmed this and demonstrate a rather efficient mechanism for the accomplishment of this end. Apparently changes of long duration are difficult to maintain. The only method whose effectiveness has been adequately demonstrated is that of increasing the acidity by the administration of large amounts of lactose. Several studies have been made of this process.[2, 3]

* Aided by a grant from the Division of Medical Science of the Rockefeller Foundation.

† Fellow of the Henry Strong Denison Foundation.

1 Robinson, C. S., *J. Biol. Chem.*, 1935, **108**, 403.

2 Robinson, C. S., Huffman, C. F., and Mason, M. F., *J. Biol. Chem.*, 1929, **84**, 257.

3 Robinson, C. S., and Duncan, C. W., *J. Biol. Chem.*, 1931, **92**, 435.

Presumably it depends on the alteration of the intestinal flora in such a way that the acid-producing organisms predominate and by the production of lactic acid produce their effects. Lactose, and to a lesser extent dextrine, are the only 2 substances which accomplish this. Most of the results have been secured on carnivorous or on omnivorous animals like the dog, cat and rat. The ordinary alpha lactose has always been used.

It occurred to us that in an herbivorous animal with a longer intestinal tract the effects of lactose feeding should be emphasized by the longer time required for the passage of material through the gut. It was also thought that beta lactose, whose nutritional status has become of interest since processes for its manufacture have made it available might, because of its greater solubility, be less effective than the alpha form.

The general plan was that followed in previous work. Three groups of about 15 rabbits each were fed the same basal ration, 40% alfalfa, 40% oats to which was added 20% dextrose, alpha lactose or beta lactose respectively for each group. They were maintained on these diets for at least two weeks and then killed. The intestines were immediately dissected out, tied off into sections (five for the small intestine between the pylorus and ileocecal valve, one for the cecum and 2 for the colon) and the reaction of the contents determined. The measurements were made electrometrically with the quinhydrone or glass electrode. Table I shows the differences of the means and the probable errors of these differences.

The results were unexpected for several reasons. Thus, contrary to previous results, the alpha lactose produced a higher pH in the small intestine than did the dextrose although the differences were not generally significant. In rats this happened only with diets high in animal protein.[3] In the cecum and colon the normal relationship was restored and the lactose-fed rats produced the more acid

TABLE I.

Section of Intestine	Dextrose and α-Lactose			Dextrose and β-Lactose		
	Means Dex. Lac.	Mean Deviation	Probable Error	Means Dex. Lac.	Mean Deviation	Probable Error
1*	6.76-6.75	—.01	±.05	6.76-6.72	—.04	±.06
2*	6.88-6.97	+.09	±.05	6.88-6.83	—.05	±.04
3*	7.00-7.17	+.17	±.06	7.00-6.95	—.05	±.04
4*	7.20-7.37	+.17	±.05	7.20-7.06	—.14	±.05
5*	7.27-7.36	+.09	±.06	7.27-7.15	—.12	±.05
6	6.07-5.76	—.31	±.10	6.07-5.56	—.51	±.07
7	6.62-6.16	—.46	±.09	6.62-6.05	—.57	±.07
8	6.73-6.57	—.16	±.10	6.73-6.11	—.62	±.09

*Small intestine.

contents. The other outstanding feature of the picture was the effectiveness of the beta lactose in producing a more acid condition throughout the whole length of the gut.

Although in general the reaction of the contents of the rabbit's intestine is more acid than that of the rat, the differences in the large bowel between the dextrose and alpha lactose are of approximately the same order of magnitude as those found in previous work. Unfortunately a strict analogy cannot be drawn because in the work with rats dextrose was not added to the control diet, but other workers have failed to find any change under its influence. In the case of the beta lactose, however, the differences exceed those found with rats. This is apparently due to the greater effectiveness of the beta form of the sugar. It may be that its greater solubility makes it more available for bacterial action.

The unique behavior of lactose in passing through the upper intestine has usually been ascribed to its comparatively low solubility. It may, however, be partly due to the relatively small amounts of lactase in the intestines of mature animals, particularly the rabbit. The failure to split lactose into the constituent monosaccharides perhaps hinders its absorption and allows it to reach the lower section of the bowel to become available there for bacterial consumption.

We are indebted to the Borden Company for the lactose used in this work.

9562

Cutaneous Absorption of Insulin.

RALPH H. MAJOR AND MAHLON DELP.

From the University of Kansas School of Medicine, Kansas City, Kansas.

In a recent article on the cutaneous absorption of insulin Bruger and Flexner[1] came to the conclusion that "the absorption of insulin by the skin of rabbits is dependent upon the integrity of the integument. The intact skin shows little or no absorption, whereas a recently abraded skin, such as produced by shaving permits the absorption of an appreciable amount of insulin."

While there can be no doubt that actual abrasion of the skin permits absorption of insulin, there is evidence that absorption may occur under conditions where no abrasion can be detected or even

[1] Bruger, M., and Flexner, J., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 429.

suspected. In a series of some 250 individual experiments carried out during the past year, we have excellent evidence that the absorption of insulin through the skin of rabbits and of certain diabetics cannot be explained on the assumption that the procedures employed produce actual abrasions of the skin through which absorption of insulin occurred.

In all of the following observations the method employed in applying the insulin was identical. Glycerine was rubbed gently into the skin and 15 minutes later, insulin dissolved in a solution of diethylene glycol monoethyl ether was applied. The preliminary treatment of the skin, however, differed as will be indicated in the different experiments.

If the simple shaving of the rabbit's skin produced abrasions sufficient to permit insulin absorption, we should expect a marked fall in blood sugar almost uniformly if the application of insulin follows the shaving immediately. The following typical observation shows that this is not the case.

Rabbit No. 46. Insulin applied to abdomen 15 minutes after shaving.

Time	Blood Sugar	Remarks
9:45 A.M.	105	Insulin 25 units applied at 10:00 A.M.
11:45 "	108	
1:25 P.M.	114	
3:00 "	115	

Similar results were obtained in 28 experiments.

In the following group of experiments the rabbits were shaved 24 hours before the application of glycerine and insulin. We did not carry out any experiments as did Bruger and Flexner in which 6 and 7 days elapsed between the time of shaving and that of applying insulin, since our animals during this period developed a new growth of hair which we considered sufficient to interfere with the absorption of insulin.

Rabbit No. 38.

Time	Blood Sugar	Remarks
9:45 A.M.	134	10 units insulin applied 10:00 A.M.
11:35 "	59	
1:25 P.M.	103	
2:50 "	121	

Rabbit No. 49.

Time	Blood Sugar	Remarks
9:55 A.M.	175	10 units insulin applied 10:00 A.M.
11:50 "	67	
1:35 P.M.	104	
3:10 "	103	

Ten similar results were obtained.

Hermann[2] has laid great stress upon alkalinization of the skin before the application of insulin. In 5 experiments on rabbits, the skin of the abdomen, which had been shaved 24 and 48 hours before, was treated with a 1% solution of NaOH which was washed off in 5 minutes and then glycerin and the insulin solution applied. In all of these experiments a marked fall in blood sugar occurred.

The observations upon diabetic patients show even more strongly that abrasion of the skin is not the determining factor in insulin absorption from the skin. Thirty-four different observations were carried out. The insulin was applied to the anterior aspect of the thigh after preliminary treatment with glycerin. The area was not shaved.

In this group of 34 observations, 16 showed no change, 3 showed a fall of 20 mg., 6 a fall of 30 mg., 2 a fall of 50 mg., one a fall of 60 mg., 3 a fall of 70 mg., one a fall of 80 mg., and 2 a fall of 110 mg. The dose of insulin employed was 25 units and 50 units. The 2 following protocols are examples of observations where a marked fall in blood sugar occurred.

M.P. age 23 severe diabetes mellitus.

7/19/37	fasting blood sugar 282	7/11/37	307
	25 units of insulin applied after drawing blood		
	Blood sugar after 1 hr. 250		280
	" " " 2 " 210		250
	" " " 3 " 178		200
	" " " 4 "		204

A.C. age 40 severe diabetes mellitus.

5/18/37	25 units applied after drawing blood	
	Blood sugar after 1 hr. 210	
	" " " 2 " 195	
	" " " 3 " 189	

The fall in blood sugar in these patients could not have been caused by skin abrasions due to shaving since this procedure was not carried out. In these observations it should be stressed that in only slightly more than 50% was any definite fall in blood sugar observed. Also that in less than one-third was the fall 50 mg. or more. It was further observed that the same patient might on one day show a fall of 50 mg. or more and on a succeeding day prove quite refractory.

Conclusions. Cutaneous absorption of insulin occurs in rabbits and in patients independent of abrasions produced on the skin. This absorption is, however, very inconstant.

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2 Hermann, Siegwart, *Arch. f. exp. Path. u. Pharm.*, 1935, **179**, 529.

9563

Further Observations on Effects of Various Fruits on Intestinal Flora of White Rats.

Louis Weinstein and James E. Weiss. (Introduced by L. F. Rettger.)

From the Department of Bacteriology, Yale University.

Weinstein and Weiss[1] reported that banana, apple and raisins when fed to white rats subsisting on a high-protein diet consisting solely of raw chopped lean beef are able to bring about a change in the intestinal flora from a type in which non-aciduric bacteria predominate to one in which *L. acidophilus* is the outstanding organism. It was our purpose in the work presented here to further enlarge the group of fruits studied and to determine to what extent the ability to alter the intestinal flora is a general property of the fruit family or whether it is limited to certain members.

The bacteriological methods and feeding procedures used in the experiments to be described here are the same as those reported previously by Weinstein and Weiss. All of the animals were fed 12 gm. of lean chopped beef daily until repeated stool examinations revealed the absence of *L. acidophilus* from the intestine. At this time the fruit to be tested was added to the meat diet of one group of animals in 4 gm. amounts daily and 6 gm. to the diet of another group. Periodic fecal examinations were made and the *L. acidophilus* content of the feces determined.

The fruits used were cranberry, strawberry and tomato, supplied to us in the dehydrated form by a commercial concern engaged in their preparation.

Ten rats were fed cranberry-powder. One group of 5 rats received 12 gm. of meat plus 4 gm. of the powder; another was fed 12 gm. of meat with 6 gm. of the dried fruit. Two rats were used as controls; they subsisted on a diet of lean chopped beef with no added carbohydrate. Four of the 5 rats receiving 4 gm. of dried fruit underwent a change in their intestinal flora after 2 weeks of feeding. The animals were found to harbor *L. acidophilus* in quantities ranging from 50 to 95% of the total viable intestinal organisms. The fifth animal did not show any change in the fecal bacteria. Four of the 5 animals receiving 6 gm. of the dried fruit also showed a change in their intestinal flora from a predominantly non-aciduric type to one in which *L. acidophilus* was found to be the predominating or-

[1] Weinstein, Louis, and Weiss, James E., *J. Infect. Dis.*, 1937, **60**, 1.

ganism. The percentage of *L. acidophilus* in the intestine of these animals varied from 20 to 90% of the total viable organisms. The fifth animal showed no change in the type of bacteria present in the intestine when fed the dried fruit for the period equivalent to the time required to alter the intestinal bacteria of the 4 other animals. The lower percentages of *L. acidophilus* present in the animals subsisting on the larger amounts of the dried fruit cannot be explained at present. The controls which were fed lean chopped beef alone showed no *L. acidophilus* during the entire experiment.

Ten animals were fed dried cranberries; 5 received 4 gm. of the fruit, in addition to the 12 gm. of meat, while the remaining rats were fed 6 gm. of cranberry in addition to their basal ration. All of the animals receiving cranberry were found to have undergone a change of their intestinal flora to a type in which *L. acidophilus* was numerically predominant within 3 weeks. In the group which was given 4 gm. of the dried fruit the percentage of *L. acidophilus* ranged from 10% to 60%. In those receiving 6 gm. of the fruit, it was found to constitute 60 to 90% of the total viable intestinal bacteria. The controls which received only chopped meat with no added fruit showed no *L. acidophilus* in their intestine throughout the course of the experiment.

Ten animals were fed 4 and 6 gm. of tomato-powder. Although the experiment was allowed to continue until the tomato had been fed in the diet for a period of 8 weeks, no changes were observed in the intestinal flora of these animals. *L. acidophilus* could not be detected in the experimental or control animals at any time during which the feeding took place.

From the results given above it can be concluded that other fruits than banana, apple and raisins have the property of altering the intestinal flora of white rats. The reasons for the failure of tomatoes to alter the intestinal bacterial picture are not apparent at the moment.

9564

The Antigonadotropic Factor. Reversibility of the Prolan-Antiprolan Effect.

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In order to explain the mechanism of the antigonadotropic function we thought it necessary to examine the following questions: Is prolan irrevocably destroyed by antiprolan or is the process reversible? While investigating the chemical properties[1] of antiprolan we found that prolan and antiprolan differ from each other as follows: Prolan is more sensitive to HCl than antiprolan but less sensitive to NaOH. n/10 up to n/15 HCl destroys prolan, but not antiprolan; n/10 up to n/15 NaOH destroys antiprolan, but not prolan. If the prolan-antiprolan effect is reversible it must be possible to destroy antiprolan by means of NaOH and thereby to release the prolan and render it biologically active once more. By using HCl we should be in a position to destroy the prolan, to liberate the antiprolan once more and to render it capable of again inactivating freshly added prolan. If the process is irreversible the prolan-antiprolan-complex cannot be changed in any way whatsoever either by NaOH or by HCl.

The following experiments make it evident that the prolan-antiprolan effect is reversible.

The reactivation experiments can be divided into two groups. I. Reactivation of the prolan out of the prolan-antiprolan complex by selective destruction of the antiprolan by means of NaOH. II. Reactivation of the antiprolan out of the prolan-antiprolan complex by selective destruction of prolan by means of HCl.

I. *Reactivation of Prolan.* Thirty mg. of antiprolan-acetone-dry powder (corresponding to 30 PAU*) was dissolved in 1.2 cc. of aqua dest. 0.8 cc. = 20 PAU of this solution was mixed with 20 RU of prolan (dissolved in 0.4 cc. aqua dest.). This neutral prolan-antiprolan mixture (= 1.2 cc.) was now divided into 2 parts (0.6 cc. each) with which Experiments 1 and 2 were carried out. Experiments 3 and 4 served as controls.

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1 Zondek, B., and Sulman, F., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 198.

* 1 PAU = 1 prolan-anti-unit is the smallest amount of antiprolan required to annihilate the gonadotropic effect of 1 RU of prolan in a test rat. Ten PAU must be tested at least in 1 rat. *cf.* Zondek, B., and Sulman, F., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 708.

1. One-half (0.6 cc. prolan-antiprolan mixture) was placed into the incubator for 2 hours to enable union to take place. Then 2.0 cc. n/10 NaOH was added in order to release and destroy the antiprolan.

2. The other half (0.6 cc. of the prolan-antiprolan mixture) was also placed into the incubator for 2 hours to enable union to take place. Then for analogous reasons 2 cc. of m/10 NaCl solution was added. This control should prove that the prolan-antiprolan mixture neutralizes itself to complete ineffectiveness.

3. 10 PAU of antiprolan (0.4 cc.) was diluted with 0.2 cc. m/10 NaCl solution, placed into the incubator for 2 hours, and then 2.0 cc. n/10 NaOH was added. This control should prove that the antiprolan is destroyed at this concentration of NaOH.

4. 10 RU of prolan (in 0.2 cc. of aqua dest.) was mixed with a solution of 10 mg. of acetone-dry-powder of normal rabbit serum (in 0.4 cc. of aqua dest.). This mixture was placed in the incubator for 2 hours, then 2 cc. of n/10 NaOH was added. This control should prove that the gonadotropic factor was not destroyed by the concentration of NaOH applied in the presence of 10 mg. of serum protein.

The 4 tubes were kept at room temperature for 20 hours. Then 2 drops of brom-thymol-blue-indicator solution were added to each, and tubes Nos. 1, 3, and 4 were neutralized with several drops of normal hydrochloric acid. Tube No. 2 remained neutral. To tube No. 3 we added once more 10 RU of prolan. Then all 4 tubes were placed again in the incubator for 2 hours and finally the contents of each tube was injected into an infantile female rat. (The technique of the experiments has been reported upon in our previous papers.[2]) The result was as follows:

Rat 1 showed the gonadotropic reaction HVR I-III, the antiprolan having been destroyed by NaOH and the prolan thus rendered effective.

Rat 2 showed no reaction whatsoever, prolan and antiprolan having neutralized each other.

Rat 3 showed the gonadotropic reaction HVR I-III, the antiprolan having been destroyed by NaOH, the subsequently added prolan now being able to take effect.

Rat 4 showed the gonadotropic reaction HVR I-III, the prolan not having been affected by NaOH.

This experiment, as well as that following, can be reproduced at any time, if the dilutions indicated are strictly adhered to. If either

[2] Zondek, B., and Sulman, F., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 198.

the acid or the alkali is too concentrated, both prolan and antiprolan will be destroyed. If too dilute one or the other will not be completely destroyed. In order to be certain that the prolan was rendered completely inactive we used in some of the experiments an excess of antiprolan; we neutralized 10 RU of prolan with 20 or 30 RU of antiprolan. In this case too we succeeded in reactivating the prolan by selective destruction of the antiprolan by NaOH.

II. *Reactivation of Antiprolan.* Thirty mg. of acetone-dry-powder (corresponding to 30 PAU of antiprolan) was dissolved in 1.5 cc. of aqua dest. and neutralized with 30 RU of prolan (dissolved in 0.3 cc. of aqua dest.). The neutral mixture (totalling 1.8 cc.) was placed in the incubator for 2 hours to enable a complete union to take place. Then the mixture was divided into 3 parts (0.6 cc. each) and Experiments 1-3 were carried out.

1. To 0.6 cc. of the mixture 3 cc. n/10 HCl was added in order to release the 10 RU of prolan from the complex and to destroy it. Twenty hours later 10 RU of prolan was added in order to combine with the released antiprolan (see below).

2. To 0.6 cc. of the mixture (for reasons of control) 3 cc. m/10 NaCl was added. This experiment should prove that the prolan is completely inactivated by the adequate amount of antiprolan.

3. For reasons of control as well 3 cc. m/10 NaCl was also added to 0.6 cc. of the mixture and 20 hours later 10 RU of prolan was added in order to demonstrate that there was no excess of antiprolan in the mixture (see below). As a further control Experiments 4 and 5 were carried out.

4. Ten PAU of antiprolan (dissolved in 0.6 cc. of aqua dest.) was added to 3 cc. of n/10 HCl, kept at room temperature for 20 hours and subsequently neutralized with some drops of n/NaOH. Then 10 RU of prolan was added to demonstrate that the antiprolan had not been destroyed by the produced concentration of hydrochloric acid and, therefore, was able to inactivate the 10 RU of prolan (see below).

5. Ten RU of prolan (dissolved in 0.6 cc. of aqua dest.) was mixed with 10 mg. acetone-dry-powder from normal rabbit serum (in 0.5 cc. of aqua dest.). To this mixture we added 3 cc. n/10 HCl and after 20 hours' contact at room temperature neutralized with normal NaOH. This control should demonstrate that the produced concentration of hydrochloric acid destroys the prolan if serum protein is present. The 5 tubes were allowed to stand at room temperature, as described above, in order to enable the hydrochloric acid to take effect. Then 2 drops of brom-thymol-blue-indi-

cator solution were added to every tube. Tubes Nos. 2 and 3 were neutral. Tubes Nos. 1, 4, and 5 were neutralized with some drops of NaOH. Then—as already mentioned above—10 RU of prolan was added to tubes 1, 3, and 4. Finally all 5 tubes were placed once more in the incubator for 2 hours, and the contents of each tube was injected into an infantile female rat. The result was as follows:

Rat 1 showed no gonadotropic reaction whatsoever, for the prolan which had been added at first had been destroyed by the HCl. That is why the subsequently added 10 RU of prolan could be inactivated once more by the released reactivated antigonadotropic factor.

Rat 2 showed no gonadotropic reaction whatsoever, for the 10 PAU of antiprolan and the 10 RU of prolan had annihilated each other completely.

Rat 3 showed the gonadotropic reaction HVR I-III, for by the *second* addition of 10 RU of prolan an excess of gonadotropic factor had been produced which could no longer be bound. The 10 antiprolan units present had been quantitatively bound by the first 10 RU of prolan.

Rat 4 showed no gonadotropic reaction whatsoever; the previously used 10 PAU of antiprolan not having been affected by the hydrochloric acid was thus still able to neutralize the subsequently added 10 RU of prolan.

Rat 5 showed no gonadotropic reaction whatsoever, for the 10 RU of prolan contained here had been destroyed by the hydrochloric acid.

Basing upon our previous investigations we arrive at the following conclusions regarding the mechanism of the antigonadotropic function:

Antiprolan is not a hormone *strictu sensu*. It is to be expected from an (anti)hormone that it can be demonstrated in the blood under normal conditions. We have not yet succeeded in doing so with antiprolan.

We demonstrated[2] that antiprolan is still effective if injected into animals 8 days previously to prolan. Consequently antiprolan maintains its effectiveness in the blood for 7 days. Hitherto no hormone is known which remains active in the blood for such a lengthy period, if applied in aqueous solution subcutaneously. Furthermore, our investigations[3] demonstrate that there is a high degree of species and organ specificity (99.5 and 93%) which also speaks against the hormonal character of antiprolan.

Is antiprolan a ferment? For neutralizing prolan a constant

[3] Zondek, B., and Sulman, F., Proc. Soc. Exp. Biol. and Med., 1937, **36**, 713.

amount of antiprolan is necessary; consequently prolan and antiprolan quantitatively neutralize each other. The time of the reaction of prolan with antiprolan does not affect the result. This fact suggests the assumption that antiprolan is not a ferment, as does the fact that prolan and antiprolan can be reactivated out of the prolan-antiprolan complex.

Is antiprolan an antibody? The serological investigations performed hitherto do not favor the assumption of an antibody, for complement fixation, precipitation and skin reactions cannot be performed with a serum rich in antiprolan, provided that the animals had been "immunized" with a pure prolan.[4] The antigonadotropic reaction certainly does not belong to that group of immune-reactions which are unmistakably provable by serological methods.[4-9]

At any rate we find especially among the toxins functions similar to those of antibodies but without the presence of serologically recognizable antibodies. It was not until the application of Ramon's flocculation method (which allows the toxins and antitoxins to flocculate—under optimal quantitative conditions—in the water-bath at 45°C.) that we knew that there were substances similar to the antibodies, the strong activity *in vivo* being easily provable but of which the serological proof *in vitro* may only be achieved under especially controlled conditions. We did not succeed with the help of Ramon's method in bringing about flocculation in the antigonadotropic serum after having added purified prolan.

While hitherto all serological reactions of antiprolan were negative we succeeded, however, by protracted treatment of rabbits with gonadotropic hormone from mare's serum (prosylan) in demonstrating spasmodically occurring evanescent antibodies which gave the complement fixation. One of us (S.) will report in detail upon this elsewhere. We mention it here in order to demonstrate that although by the serological methods used hitherto the antiprolan cannot be identified as an antibody and prolan as an antigen, this negative finding does not prove the antiprolan reaction not to belong to the group of the immune-biological reactions. In any case the following findings favor the assumption of an immune-biological reaction:

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4 Sulman, F., *J. Exp. Med.*, 1937, **65**, 1.

5 Twombly, *Endocrinology*, 1936, **20**, 311.

6 Brandt, R., a. Goldhammer, H., *Z. Immunitatsf.*, 1936, **88**, 79.

7 Bachmann, C., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 851.

8 Gustus, E. L., Meyer, R. K., a. Dingle, J. H., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 257.

9 Eichbaum, F., a. Kindermann, V., *Z. Immunitatsf.*, 1935, **86**, 284.

1. The injection of antiprolan protects a rat for 8 days against the effect of prolan. This process especially points to a passive immunization.

2. Antiprolan is not to be found in the albumin but in the globulin of the serum, namely in certain fractions of the globulin (pseudoglobulins)[2] thus resembling some of the antibodies.

3. The reversibility of the antiprolan effect also speaks in favor of an immune-biological reaction.†

While our experiments suggest that antiprolan is neither a hormone-like nor a ferment-like body, yet on the other hand we find some, if not all, properties corresponding to those of an immune-biological body. It is possible that here we have to deal with a new kind of body approaching very closely the immune-bodies without producing the vitro-reactions typical of those bodies.

Summary. The inactivation of prolan by antiprolan is a reversible process, since prolan and antiprolan may be released from a neutral prolan-antiprolan mixture and thereby reactivated.

The assumption is made that antiprolan is neither an (anti)hormone *strictu sensu* nor a ferment, but is possibly a new kind of factor approaching very closely the immune-bodies to which it is in some respects quite similar.

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Effect of Vitamin D on Growth of Tubercle Bacilli.*

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It was shown[1, 2] that feeding irradiated cream or oil concentrates moderately high in vitamin D to tuberculous guinea pigs produced no change in the course of the disease.

To control this observation further it was decided to observe the

† In a previous report[3] we expressed the supposition that antiprolan destroys prolan. The experiments reported here, however, show that this supposition was wrong for now we can prove the reversibility of the reaction.

* This study was made possible through a grant from the Wisconsin Alumni Research Foundation.

1 Loewen, David F., and Oatway, Wm. H., *Am. Rev. Tuberc.*, 1936, **33**, 733.

2 Steenken, Wm., Jr., and Baldwin, E. R., *Am. Rev. Tuberc.*, 1937, **35**, 656.

effect of the addition of crystalline vitamin D in propylene glycol† upon the growth and virulence of variants from the same parent strain H_{37}.

To small flasks containing 20 cc. of Proskauer and Beck's synthetic medium‡ crystalline vitamin D solution was added in 4 amounts ranging from 0.05 to 0.2 cc. (100 to 400 U.S.P. units). As a control propylene glycol was added in the same concentrations to the basic medium.

Control and experimental media were seeded with Ra and Rv[3] variants of the H_{37} strain. The fully virulent Rv variant was the organism used for infecting all animals in the previous experiments. Both variants were carried through 3 generations on the 2 media, being transferred at 21-day intervals. Growths characteristic of each variant were observed in each generation. The Rv growth was veil-like and spreading whereas the Ra was heaped up and dense with a clear-cut periphery.

At the end of the third 21-day interval of transfer, suspensions were made of the organisms from each flask so that one cc. contained 0.5 mg. dry weight. Each of these suspensions was divided into 2 parts. One part was plated on gentian-violet-egg media for colonial study. At the end of 6 weeks, the colonies were compared and no alteration of their original form was discovered. The other part was divided and inoculated intratesticularly into 2 guinea pigs.

Forty days after inoculation a control pig (Rv) died; its lungs, liver, spleen and glands were riddled with tuberculosis.

On the same day one of each pair was killed. All of the animals receiving the Rv variant had generalized tuberculosis, whereas all of the pigs receiving the Ra variant had only local caseous lesions in the testicle.

Fifty days from the date of inoculation an Rv pig (200 vitamin D units) died of generalized tuberculosis. All remaining Ra and Rv pigs were killed and pathological changes similar to those in previously reported series were observed.

Summary. Crystalline vitamin D (100-400 units) when added to Proskauer and Beck's medium did not change the virulence or

† Supplied to us through the courtesy of Winthrop Chemical Co. (10,000 U.S.P. units per gm.).

‡ Monopotassium phosphate 5.0 gm.
Asparagin 5.0 ''
Magnesium sulphate 0.6 ''
Magnesium citrate 2.5 ''
Glycerol 20.0 cc.
pH 7.4

3 Steenken, Wm., Jr., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 253.

growth characteristics of either the Ra or Rv variants of strain H_{37} *M. tuberculosis.*

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Nitrogen, Sulfur, Sodium, Potassium and Chloride Metabolism in Vitamin B_1 Deficient Rats.

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Metabolic studies in vitamin B_1 deficient animals have been largely limited to studies on carbohydrate metabolism and respiration of the central nervous system and other tissues.

To our knowledge no studies on the metabolism of the usual food elements and electrolytes have been made in vitamin B_1 deficient animals.

Twenty-four male and female albino rats (Wistar strain) were used in the experiments. They were kept in groups of 4 in metabolism cages, as previously described.[1] The diet consisted of purified casein 18%, Osborne-Mendel salt mixture 4%, butterfat 8%, cod liver oil 2%, corn starch 53%, and autoclaved baker's yeast 15%. In order to be able to differentiate clearly between the effects of vitamin B_1 deficiency and of inanition, paired feeding was resorted to. The food consumed by the controls consisted of the same ingredients as that given to the experimental animals, except that unautoclaved yeast was used. They received an amount of food corresponding to that eaten by the experimental animals during the preceding week. Distilled water *ad lib.* was provided from automatic glass fountains.

Urine and feces were collected twice a week. The urine was analyzed for total nitrogen (Kjeldahl), urea,[2] ammonia (Folin), uric acid,[3] creatine and creatinine,[4] total sulfur,[5] total and inorganic sulfates (Folin), sodium and potassium,[6] and chloride (modified Volhard-Harvey method). Feces were analyzed for total nitrogen, total sulfur,[7] and chloride (open Carius method). Food was analyzed

1 Sandberg, M., and Perla, D., *J. Exp. Med.*, 1934, **60**, 395.
2 Van Slyke, D. D., and Cullen, G. E., *J. Biol. Chem.*, 1914, **19**, 211.
3 Benedict, S. R., and Franke, E., *J. Biol. Chem.*, 1922, **52**, 387.
4 Folin, O., *J. Biol. Chem.*, 1914, **17**, 469.
5 Benedict, S. R., *J. Biol. Chem.*, 1909, **6**, 363.
6 Smith, G. F., and Ross, G. F., *J. Am. Chem. Soc.*, 1925, **47**, 1020.
7 Neumann, A., and Meinertz, J., *Z. physiol. Chem.*, 1904-5, **43**, 37.

for total nitrogen, total sulfur, sodium, potassium and chloride. In order to conserve space condensed protocols for nitrogen and sulfur metabolism only will be presented, since there is no change in the excretion of sodium, potassium and chloride.

TABLE I.
Nitrogen Metabolism in Vitamin B_1 Deficient Rats and Paired Feeding Controls. Daily Average.

	Total N							% of total urinary N excretion
	Urine mg.	% of Intake	Feces mg.	% of Intake	Intake mg.	Retention, mg.	% of Intake	Urea N
Vitamin B_1 deficient rats								
Control period								
Oct. 6-Nov. 16	97	26	57	15	374	220	59	78
Deficiency period								
Nov. 17-Dec. 28	108	56	25	12	200	67	32	79
Paired feeding controls								
Control period								
Oct. 13-Nov. 23	71	19	47	12	364	246	69	74
Inanition period								
Nov. 24-Jan. 4	59	28	25	12	210	126	60	82

TABLE II.
Sulfur Metabolism in Vitamin B_1 Deficient Rats and Paired Feeding Controls. Daily Average.

	Total S						
	Urine mg.	% of Intake	Feces mg.	% of Intake	Intake mg.	Retention, mg.	% of Intake
Vitamin B_1 deficient rats							
Control period							
Oct. 6-Nov. 16	9.6	21	8.2	18	44.9	27.1	61
Deficiency period							
Nov. 17-Dec. 28	9.9	48	4.1	19	21.1	7.1	33
Paired feeding controls							
Control period							
Oct. 13-Nov. 23	6.7	15	6.9	16	43.3	29.7	69
Inanition period							
Nov. 24-Jan. 4	6.1	24	4.2	17	24.9	14.6	59

In vitamin B_1 deficient rats there is a disturbance in protein metabolism. Urinary nitrogen excretion increases in the vitamin B_1 deficient animals from 26% of the intake during the control period to 56% during the deficiency period. The paired feeding controls show an increased urinary nitrogen excretion rising from 19 to only 28% of the intake. Apparently, the increased urinary nitrogen excretion is only in part due to inanition, and to some extent to a specific factor in vitamin B_1 deficiency. The rise in nitrogen ex-

cretion is accounted for entirely by urea nitrogen, for there is no change in the excretion of ammonia nitrogen, creatinine, creatine, or uric acid. Fecal nitrogen excretion falls with the decreased intake in the vitamin B_1 deficient animals as well as in the paired feeding controls.

Total urinary sulfur excretion shows nearly the same absolute value during the deficiency as during the control period, but expressed in percent of intake it parallels the excretion of nitrogen, rising from 21 to 48%. There is hardly any change in the total sulfates as expressed in percent of the total urinary sulfur excretion. The rise in neutral and ethereal sulfur, indicating a disturbance in endogenous sulfur metabolism, is the same as that observed in the paired feeding controls. Fecal sulfur excretion drops with the decreased intake, but expressed in percent of intake it remains unchanged.

It is apparent that the only significant disturbance in metabolism in vitamin B_1 deficient animals is in the increased urinary nitrogen and sulfur excretion which exceeds, to a considerable extent, the increased excretion of nitrogen and sulfur observed in the paired feeding controls.

Though vitamin B_1 is significant in oxidative processes in cells of the central nervous system and possibly in cellular metabolism in general, its complete depletion is not associated with disturbances in the metabolism of most of the essential elements of the diet.

9567

Presence of an Antagonistic Factor in Serum of Dogs Following Repeated Injections of Cortin.*

C. GWENDOLINE TOBY AND LENA A. LEWIS. (Introduced by F. A. Hartman.)

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It has been reported[1] that in the normal dog a marked decrease in the excretion of Na and usually an increase in the excretion of K occurs during a 6-hour period following the intravenous injection of 20-40 cat units of cortin; and that with repeated injections (at

* Aided by a grant from the Rockefeller Foundation administered by Dr. F. A. Hartman.

1 Hartman, F. A., Lewis, L. A., and Toby, G., *Science*, 1937, **86**, 128.

intervals of several days), the effect becomes progressively less, until there is little change in electrolyte excretion following administration of the extract. It has since been found that NH_3 excretion is also increased following cortin and that with repeated injections this response is decreased. Of 9 animals injected with 20-40 cat units of cortin, all gave the characteristic electrolyte response described (Table I). Four different cortical extracts prepared in this laboratory were used. The response elicited by a given extract in different dogs was very similar (Table I). Five of these animals were injected until they became completely refractory.

TABLE I.
Initial Response to Intravenously Injected Cortin in the Normal Female Dog.

Dog	Treatment	m. eq. excreted in 6 hr. Na		K	NH_3-N	
I	Controls (6)	8.71	(6.18-10.18)*	5.11	6.35	(2)
	Cortin 1	1.24		6.25	11.00	
III	Controls (4)	10.51	(7.64-14.01)	8.31	5.75	(2)
	Cortin 1	1.15		8.62	12.50	
IV†	Controls (3)	9.76	(8.25-11.21)	8.64	5.20	(2)
	Cortin 1	3.21		8.36	9.40	
V	Controls (2)	12.50	(11.30-13.69)	5.56	6.35	(1)
	Cortin 2	6.65		6.05	7.93	
VIII	Controls (2)	15.55	(13.67-17.43)	6.20	6.35	
	Cortin 2	6.98		6.43	12.11	
XIII	Controls (4)	17.33	(15.00-19.44)	9.12	7.28	(2)
	Cortin 2	11.86		9.84	10.65	
XIX	Controls(4)	12.23	(10.24-13.80)	7.48	7.14	
	Cortin 3	6.80		8.60	13.00	
VII	Controls (3)	15.71	(13.82-19.29)	6.79	5.53	
	Cortin 4	7.98		7.81	7.43	
Average change after cortin		—7.05		+0.60	+4.26	

*range.
†one previous injection.

Failure of these animals to respond to further injections suggested the possibility that some antagonistic substance was present in the blood. Using sterile precautions, blood was taken from 2 dogs (I and III), which had received 7 and 11 injections respectively over periods of 5 and 9 weeks. The serum was separated and stored at 4°C. Five untreated animals were given 10-25 cc. of this serum intravenously about 15 minutes previous to the injection of the usual amount of cortin. In every case in which cortin and serum were given, the Na and the NH_3 did not change beyond the range of the controls, whereas cortin alone always reduced the Na to a level well below that of the lowest control and increased the NH_3 well above the highest control value (Table II). Decreases in Na of 86-89% in 3 animals (I, III, X) were shown when 0.5 cc. (20

C.U.) of extract 1 was given. The same amount of this extract given with serum from refractory dogs decreased the Na excretion by only 14 and 17% (XI, XII). Extract 2 (40 C.U.) gave decreases of 32-55% in 3 animals (V, VIII, XIII). When injected following serum, the same amount of this extract gave, in one instance, an increase of 5% over the control levels (XIV) and in another, a decrease of 1% (XV). In one case where only 10 cc. of the serum was used with extract 2, there was a decrease of 17% in Na excretion (XVI). The injection of serum from normal dogs had no inhibitory effect (IX and X). Since the range for K and NH_3 was small and comparable with the range of Na these values are not included in the table.

TABLE II.
Initial Response to Intravenously Injected Serum and Cortin in the Normal Female Dog.

		m. eq. excreted in 6 hr.		
Dog	Treatment	Na	K	NH_3-N
X	Controls (4)	11.17 (7.14-16.37)*	6.60	6.50
	Last control before inj.	10.60	5.95	7.55
	Normal serum—18 cc.			
	Cortin 1	2.15	6.70	10.10
IX	Controls (2)	6.30 (5.90-6.70)	5.21	5.58
	Last control before inj.	5.90	5.09	5.58
	Normal serum—20 cc.			
	Cortin 3	3.04	5.85	7.45
XI	Controls (2)	9.61 (6.04-13.19)	5.57	5.92
	Last control before inj.	6.04	6.04	6.00
	Sera I and III—23 cc.			
	Cortin 1	7.98	5.52	6.56
XII	Controls (3)	16.35 (11.96-20.70)	9.26	5.50
	Sera I and III—18 cc.	13.98	8.68	5.57
	Cortin 1			
	Control after inj.	11.96	10.16	6.50
XIV	Controls (3)	11.87 (9.46-14.08)	8.12	9.80
	Last control before inj.	12.08	8.41	9.06
	Sera I and III—25 cc.			
	Cortin 2	17.90	9.20	7.35
XV	Controls (4)	11.56 (11.06-11.90)	6.73	6.35
	Last control before inj.	11.06	6.17	6.42
	Serum I—25 cc.			
	Cortin 2	11.43	6.98	6.35
XVI	Controls (8)	9.38 (7.34-11.67)	6.59	7.50
	Last control before inj.	7.92	6.30	8.15
	Sera I and III—10 cc.			
	Cortin 2	7.80	7.00	7.70
Aver. change after serum and cortin		+0.34	+0.22	—0.35

*range.

Serum obtained from dogs I and III several weeks after the last injection of cortin still demonstrated this inhibitory effect. Storage at 4°C. for 2-3 weeks did not destroy the antagonistic factor.

The methods for analyses of Na and K were those previously described.[2] NH_3 was determined by Sobel, Yuska and Cohen's modification of Van Slyke and Cullen's method.

The cortical extracts used were highly purified, contained very little solid, were free from protein and contained practically no nitrogen. Potent extracts gave a precipitin reaction with serum from refractory dogs, but did not react with normal dog serum. Preliminary experiments, using cortin as the antigen, would indicate that the serum of repeatedly injected animals may have some power of fixing complement.

We wish to express our thanks to Dr. F. A. Hartman for his helpful criticism and advice throughout the course of these experiments.

9568 P

Determination of Amino-Nitrogen in Urine.

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Despite the importance of an understanding of amino-acid metabolism, there has been no trustworthy method of amino-nitrogen determination that could be easily applied as a routine in a clinical laboratory. The chief difficulty has been in the preparation of filtrates of blood and urine for the actual titrations. In this note a method is described for the removal of NH_3 and CO_2 from urine filtrates enabling one to determine amino-nitrogen expeditiously by the Sörensen titration. Many determinations may be carried out in parallel with little supervision by the analyst.

The Sörensen[1] titration as described by Northrop[2] is used. The preparation of urine filtrates follows the procedure of Van Slyke and Kirk.[3] Instead of distilling the NH_3 and CO_2 successively *in vacuo,* the filtrates are exposed in shallow layers *in vacuo* in a desiccator over dilute H_2SO_4. This means of collecting or removing NH_3 has been known for a long time and occasional reference is

2 Thorn, G. W., Garbutt, Helen R., Hitchcock, F. A., and Hartman, F. A., *Endocrin.*, 1937, **21**, 213.

1 Sörensen, S. P. L., *Biochem. Z.*, 1908, **7**, 45.

2 Northrop, J. H., *J. Gen. Physiol.*, 1926, **9**, 767.

3 Van Slyke, D. D., and Kirk, E., *J. Biol. Chem.*, 1933, **102**, 651.

made to it in the current literature. It requires no further effort on the part of the analyst, in contrast to the individual distillations *in vacuo* as described by Van Slyke and Kirk or as further improved by Kirk.[4] Quantitative transfer of solutions from the large inaccessible surface of the flask is likewise avoided.

In preliminary trials it was found that NH_3 could be removed from ammonium chloride in about 3 hours. With urine filtrates, however, 5-7 hours was required for constant titration figures. The alkaline filtrate remains unchanged in the refrigerator for several days so that analyses may be repeated.

Method. Urine filtrates after Van Slyke and Kirk (5 ml.) are placed in 50 ml. beakers with as little exposure to air as possible. The lips of the beakers may be paraffined to facilitate pouring. At the same time 5 ml. of the filtrates are placed in test tubes. Neutral red solution and 1 ml. of formalin are added to each tube. If the solutions remain alkaline, sufficient alkali has been added to set free the NH_3 present. Otherwise more barium hydroxide must be added to the samples in the beakers. The beakers are placed in a vacuum desiccator over broken alundum saturated with dilute (1-3) H_2SO_4.* The pressure is reduced to 20 mm. or less and the desiccator allowed to stand over night. Twelve beakers may be placed in an 8-inch desiccator.

The following morning the beakers are removed, a drop of neutral red indicator solution (0.025%) added to each and the solutions acidified with 0.1 N HCl. Two drops in excess are added to maintain a slight acidity after decomposition of a small amount of barium carbonate and release of CO_2.

The solutions are again evacuated for 1-2 hours, after which they are transferred to test tubes for titration. By using a very fine paraffined tip on the supply of CO_2-free water (discharging 1 ml. in 2.5 sec.) the solutions are removed quantitatively with less than 1.5 ml. of water—the amount lost by evaporation in the desiccator. They are thus brought to the original volume. Neutral and alkaline standards are prepared and the amino-acid solutions are titrated with 0.01 N NaOH. Formalin blank titrations are subtracted.

Especially with highly colored urines, it is difficult to prepare

[4] Kirk, E., *Acta med. Scand.*, 1936, Suppl. **77**, 46.

* The broken alundum (furnished by the Norton Co. and the Fisher Scientific Co.) is prepared by soaking in the H_2SO_4 and is renewed by pipetting the acid over its surface in the desiccator and then removing the liquid that drains to the bottom. If much free liquid is present, there is spattering on evacuation.

several neutral end point standards independently and have them match each other. As a substitute the neutral red indicator solution is diluted 5 times with water and 1 drop added to the alkaline filtrate to serve as standard. One drop of 0.01% phenol red is added and then 1.0 N HCl to neutrality, followed by 5 drops in excess The pH is then 1-2 and the color matches that of the stronger neutral red solution at pH 7. The urine filtrate may be used for this standard since the solution is acid in reaction. It replaces the Northrop neutral standard containing phosphate.

In a series of 63 determinations in triplicate, the variation has averaged 0.02 ml. with a maximum of 0.07 ml. In comparisons with titrations after distillation *in vacuo* the variation has been 0.04 ml. which is the error to be expected from duplicates by that method. Much of the variation is probably caused by inequality in size of drops of indicator solutions so that titrations carried out with independently made standards will vary more than those made with the same standards.

9569

Hydrogen-Ion Concentration of the Gall Bladder Bile of the Dog.

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An experimental study was reported[1] on the hydrogen-ion concentration of the bile of the guinea pig. In this series of experiments, effort has been made to determine which constituents of the dog's gall bladder bile are responsible for the fluctuating pH in normal dogs and also upon medication with Extract of Ox Bile, U.S.P.

The hydrogen-ion concentration of the gall bladder bile of normal dogs was determined by means of the glass electrode at 25° and an analysis of the principal constituents was carried out according to Douglas-Sauermann's[2] method. The results on 8 dogs are shown in Table I.

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* Emerson Fellow in Pharmacology.

1 Krantz, J. C., Jr., Feldman, M., Morrison, S., and Carr, C. J., Proc. Soc. Exp. Biol. and Med., 1936, **35**, 48.

2 Douglas-Sauermann, A. G., *Z. Physiol. Chem.*, 1935, **231**, 92.

TABLE I.
Constituents of Normal Dog Bile.

	pH	Total Solids, %	Ash, %	Alkalinity of Ash, % as Na_2CO_3	Bile Acids Cholic and Desoxycholic %
Aver.	6.15	23.00	1.78	.71	6.75
Low	5.78	21.85	1.66	.58	3.72
High	6.93	24.40	1.88	.86	8.37

A series of 21 dogs were fed 1.5 and 8.0 gm. of extract of ox bile per day for varying periods of time. This produced a cholagogue effect in direct proportion to the quantity of ox bile administered and the time of medication. Thus the bile becomes less acidic and lower in total solids when ox bile is fed. The bile constituents are recorded in Table II.

TABLE II.
Constituents of Dog Bile After Ox Bile Feeding.

	pH	Total Solids, %	Ash, %	Alkalinity of Ash, % as Na_2CO_3	Bile Acids Cholic and Desoxycholic %
		1.5 gm. Ox Bile Daily (25 to 72 days).			
Aver.	6.66	21.67	1.67	.69	6.09
Low	6.20	18.94	1.38	.51	3.63
High	7.35	23.86	2.08	.83	7.87
		8.0 gm. Ox Bile Daily (11 to 67 days).			
Aver.	7.24	17.56	1.66	.60	5.35
Low	6.06	8.61	1.08	.40	3.49
High	8.02	25.22	2.08	.85	7.25

In these 29 determinations the correlation coefficient between the pH and total solids is –0.83, P.E. ±0.04. The correlation coefficient between the pH and ash is –0.46, P.E. ±0.14; that between the pH and the alkalinity of the ash is –0.60, P.E. ±0.12; and that between the pH and the bile acids is –0.46, P.E. ±0.14.

These data indicate that a concentrated bile is likely to exhibit a low pH despite wide variations in its constituents, such as bile acids and ash. Kjeldahl determinations on the residue of several specimens of bile showed that the nitrogen content varied directly as the total solids. It is likely, therefore, that bile protein buffers the potential hydroxyl ions of bile and permits the acidity of the bile acids to become manifest. This concept is in accordance with the work of Aronsohn and Andrews.[3]

Conclusion. The correlation between total solids and pH of

[3] Aronsohn, H. G., and Andrews, E., Proc. Soc. Exp. Biol. and Med., 1935, **33**, 89.

dog's gall bladder bile, normal or ox bile fed dogs, is high indicating with a certainty of approximately 45% greater than sheer chance that concentrated biles will possess a greater hydrogen-ion concentration than those which are more diluted.

9570 P

Effect of Metrazol Convulsions on Brain Metabolism.*

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The wide use of metrazol in the treatment of schizophrenia has made it advisable to study the physiological changes produced by metrazol convulsions.[1] Twelve observations were made on 7 patients with schizophrenia. As seen in Table I, 7 samples of blood were collected from the femoral artery during various stages of the convulsions, 4 pairs of samples were collected simultaneously by 2 observers from the femoral artery and internal jugular vein immediately after the seizure had ceased, as was one additional sample of arterial blood. Breathing was greatly diminished during the convulsions and this was reflected in the analyses of the arterial blood, which disclosed a retention of CO_2 as well as a diminished O_2 content. Even during the first part of the seizure, as seen in Wi, 9/13, the O_2 content was diminished so that the Hb saturation $\left(\frac{O_2 \text{ content}}{O_2 \text{ capacity}}\right)$ was reduced from a theoretical normal of 95% to 84%. As the convulsions progress, the Hb saturation continues to fall so that towards the end of the seizure the saturation of Hb may be below 50% (M., 9/7). During these convulsions the patient's face is at first a dark red color. When the convulsion is completed, the anoxemia is evidenced by a leaden cyanosis. Nevertheless, as soon as unimpeded breathing is reëstablished the Hb saturation, though still reduced, is found rapidly rising towards a normal value (last 5 observations of Table I). The anoxemia, as well as the severe

* This investigation was made possible by a grant from the Child Neurology Research.

1 von Meduna, L., *Z. f. d. ges. Neur. u. Psychiat.*, 1935, **152**, 235.

TABLE I.

Patient	Date	Oxygen Content vol. %		CO_2 Content vol. %	Glucose Content mg. %		Lactic Acid mg. %		Oxygen Capacity vol. %	Hb %	Blood drawn in regard to duration of seizure
		Arterial	Venous	Arterial Venous	Arterial	Venous	Arterial	Venous			
Wi	9/13	18.97		50.31	138				22.62	84	First half
M	9/7	8.70		63.02	102		34		20.64	42	Late
Wi	9/8	16.45		44.94	129		74		23.11	71	,,
R	9/9	14.05		56.33					22.74	62	,,
M	9/11	11.17		58.37	136				22.99	49	,,
Wa	9/11	15.35		53.78	124				22.71	68	,,
R	9/13	11.43		59.43					22.74	50	,,
N	9/3	21.37	18.36	54.23 40.91	118	103	107	105			After
Wi	9/4	17.60	9.98	38.25 47.56	104	94	126	117	22.16	78	,,
M	9/4	15.93	9.94	52.30 57.19	104	92	82	70	20.58	77	,,
F	9/4	16.28	12.80	42.45 45.19	127	110	88	89	21.99	74	,,
Wa	9/8	20.43		36.06	106				22.71	90	,,

muscular effort, combine to increase blood lactic acid and blood sugar.

These convulsions, grand mal in character, cause a temporary but marked depression of cerebral functions as evidenced by amnesia, confusion, disorientation, and the elicitation of various abnormal reflexes, such as the Babinski and ankle clonus. This change in function may be attributed to the anoxemia.

The insulin therapy for schizophrenia also involves depression of cerebral functions. It is, therefore, interesting to compare the physiological mechanism of these two forms of treatment. Insulin hypoglycemia depresses cerebral metabolism by diminishing the food supply of the brain (blood sugar),[2] while metrazol achieves the same effect by decreasing the oxygen available for the combustion of this foodstuff. Thus, insulin therapy affects the brain specially, for that organ utilizes carbohydrate chiefly, while metrazol has a generalized effect on all the organs of the body, including the brain. The effect of insulin on the brain is more prolonged, while that of metrazol is more severe. However, in both cases, the depression of cerebral metabolism seems to favor the amelioration of schizophrenia.

9571 P

Chronic Adrenal Insufficiency and Pancreas Diabetes.*

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Grollman and Firor[1] have demonstrated that chronic adrenal insufficiency, induced by various methods, is primarily a disturbance of pituitary origin. The syndrome which follows, cessation of growth, failure of reproductive activity and subnormal body temperature is, according to the above workers, relieved by administration of pituitary extracts and not by cortin. Long[2] has shown that acute adrenal insufficiency caused by the removal of the adrenal gland, though accompanied by injections of cortin, ameliorates ex-

2 Himwich, H. E., Bowman, K. M., Wortis, J., and Fazekas, J. F., *Science*, 1937, **86**, 271.

* This study was aided by a grant from the National Research Council.

1 Grollman, A., and Firor, W. M., *Am. J. Physiol.*, 1935, **112**, 310.

2 Long, C. N. H., *Am. J. Med. Sci.*, 1937, **191**, 741.

perimental pancreatic diabetes. The object of the present experiments is to determine the effects of chronic adrenal insufficiency on the course of experimental pancreatic diabetes.

Eight cats were anesthetized with sodium pentobarbital and both lumbo-adrenal veins were ligated distally and proximally to the gland. At the same time the entire pancreas was removed. The animals received neither insulin nor cortin. Fluid was given *ad lib.* along with a weighed amount of food (Bovex) each day. The periods of survival were 11, 11, 12, 15, 17, 18, 20, and 98 days. The life span of these animals was definitely prolonged by the ligation, for depancreatized cats usually succumb before 8 days. At the present time we shall present a brief description of the cat which survived for 98 days. After the operation the cat exhibited a profound glycosuria, but 2 weeks later the urine was free of sugar and remained so until death. There was a marked (50%) loss in body weight. At autopsy careful examination revealed no traces of pancreatic tissue. The adrenal glands appeared degenerated. The kidney cortex was white due to increased deposition of fat, 14.8% on analysis, although the liver was free of fatty infiltration and contained the normal amount of fat, 4.5%. Two large and several small ulcers were found on both sides of the pyloric orifice of the stomach. Histological sections of the adrenal and pituitary glands are being prepared.

Biochemical studies are in progress on several animals now under observation.

9572

Effect of Male Hormone upon Uterine Motility and the Uterus.*

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The discovery of the "progesterone-like" action of several male hormone compounds in the induction of morphological changes in the rabbit's uterus suggested the possibility that such compounds might also exert a similar effect upon uterine motility.[1] While we

* Aided by a grant from the Rockefeller Foundation.

[1] Klein, M., and Parkes, A. S., *Proc. Roy. Soc. London*, 1937, **121**, 574.

were investigating this problem, our attention was called to a paper by Robson[2] in which he described inhibition of contractions of the rabbit uterus by male hormone. As our methods differ from those of Robson and give additional information, we are reporting our results. We have studied the action of testosterone propionate on the rhythmically contracting uterus of rabbits *in vivo,* using Reynold's technique[3] of the uterine fistula.

Seven adult non-pregnant female rabbits weighing between 4 and 4.5 kg. were isolated for 2 weeks or more prior to experimentation. After preparation of the Reynolds uterine fistula only 5 rabbits exhibited spontaneous motility of the oestrous type. The other animals were discarded.

Daily records were made until typical oestrous motility was obtained. The rabbits were then injected with a single dose of 2.5 to 10.0 mg. of testosterone propionate† dissolved in sesame oil. Records were taken 8 to 24 hours later. Eight hours were found to be too short a period for the hormone to be effective, so further tests were made 24 hours after treatment. In some instances 2 successive injections were necessary to alter the motility.

Seven tests for the inhibiting action of the male hormone were made on the 5 rabbits and inhibition of motility or disturbed oestrous rhythm resulted in every case. In 2 tests there resulted a disturbed rhythm or lowered height of contraction and in 5 tests complete quiescence with only an occasional contraction. Figure 1 (part of the protocol of rabbit 6) shows complete inhibition. In 6 of the 7 tests, the uterine motility returned to normal within 1 to 5 days. No corpora lutea were found in the ovaries; the follicles present were of medium and small size. During the period of quiescence resulting from the action of testosterone propionate, 3 trials were made of intravenous injections of 2 i.u. of pituitrin with failure in each case of obtaining a strong sustained contraction.

These data are insufficient for determination of the minimal amount of testosterone propionate required to halt contractions. In one instance when 2.5 mg. were injected, the height of contractions only was lowered. Since 10.0 to 20.0 mg. gave consistent results this range of dosages was used throughout. One peculiar result was obtained which should be mentioned. Rabbit 4 showing good oestrous rhythm received 10 mg. of testosterone propionate, after which the uterine contractions were completely inhibited on

2 Robson, J., *Quart. J. Exp. Physiol.,* 1937, **26**, 355.

3 Reynolds, S., *Am. J. Physiol.,* 1930, **92**, 420.

† The testosterone propionate was furnished through the kindness of Dr. E. Schwenk, of the Schering Company.

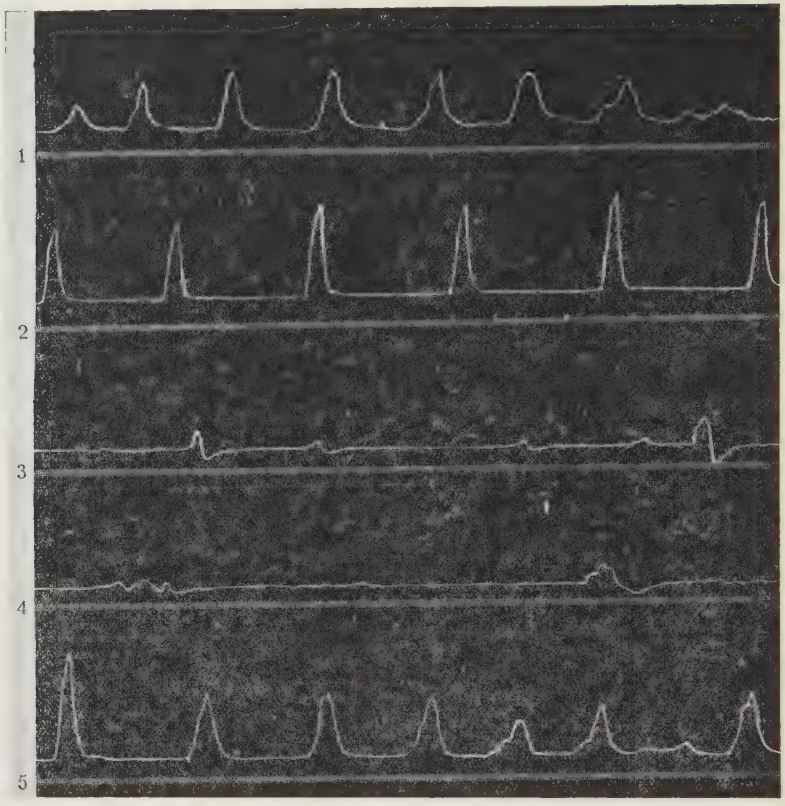

FIG. 1.

1. Good motility, typical of oestrum. Given 10 mg. of testosterone propionate.
2. 16 hours later. Contractions still regular. Given additional 10 mg. of testosterone propionate.
3. 25 hours after first injection. No motility.
4. 39 hours after first injection. No motility.
5. 5 days after first injection. Return of oestrus motility.
(Time in seconds.)

the following day. Thereupon an additional 10 mg. were given and on the next day fair motility returned but it was irregular in rhythm and unequal in height. Ten mg. more were injected and the following day the uterus was again completely inhibited and remained so for 2 days before normal oestrous motility returned.

We were also able to obtain a slight progestational modification in the uterus of young rabbits treated with testosterone propionate, confirming both Klein and Parkes[1] and Robson.[2] Six young rabbits of 2 kg. weight which had been castrated 3 weeks previously were given 70 R.U. of oestrone in 6 days. This was followed by doses of 7.5 to 20 mg. of testosterone propionate in 5 days. Microscopic

examination of the uteri in several places failed to reveal more than a ++ progestational modification and in 2 cases no modification. In all of these rabbits, however, castration atrophy of the uteri was prevented by the male hormone. Sections of the uteri of 2 of the adult rabbits used in contraction studies, which had received chronic injections of male hormone up to a few days before autopsy, showed no progestational changes. It may be added that testosterone propionate was observed to produce growth in the uterus of 3 hypophysectomized adult rats and 3 hypophysectomized and castrated adult rats.

While testosterone propionate may not be as efficient as progesterone per mg. of weight in inhibiting uterine motility, it does behave in a similar manner. Reynolds[4] found that progesterone can cause a complete inhibition of uterine contraction in the non-castrated oestrous rabbit but that the oestrous rhythm returns in 2 to 5 days after injections are stopped. This is also true of testosterone propionate. The effect of this androgen in producing uterine quiescence, however, is slower to appear than that of progesterone. Since the action of testosterone on the rabbit's uterus seems to be qualitatively similar to progesterone (though not quantitatively), there does not seem to be any evidence that 2 hormones are involved in effecting motility and progestational changes.

Summary. The male hormone, testosterone propionate, inhibited the oestrous rhythm of the rabbit uterus. This was manifested by a disturbed rhythm, a lowered height of contraction or complete quiescence. Such inhibitions appeared in animals whose uteri showed but little or no progestational response. During the period of quiescence, pituitrin failed to elicit a strong contraction. Testosterone prevented castration atrophy in the rabbit's uterus with or without minimal progestational changes.

[4] Reynolds, S., *Am. J. Physiol.*, 1932, **102**, 39.

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Changes in Nasal Mucosa of Monkeys (*Macaca rhesi*) and Humans by Male Hormone Substances.*

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A relation between certain nasal mucosal regions and sexual activity has long been known clinically,[1, 2] particularly with regard to nasal changes accompanying menstruation and pregnancy. Recently Mortimer and coworkers[3] have shown that in the monkey female sex hormones cause reddening and sometimes swelling of the "middle" and inferior conchae. The present data demonstrate that male sex substances, testosterone acetate† and testosterone propionate† effect changes in the nasal mucosa of monkeys and humans.

Thirty-one immature *Macaca rhesus monkeys* of 1.8 to 2.2 kg. were divided into the following groups which received daily subcutaneous injections:

Group	Animals	Hormone
I (a)	9 ♂	3-15 mg. testosterone acetate or propionate daily, 30 to 60 days
	4 ♀	
(b)	7 ♂	Doses graded, 1/64 mg. to 4 mg., 1-3 times weekly
II	1 ♀	3 mg. testosterone propionate and 3 mg. ketohydroxyoestrin†
III	6 ♂	Control
	4 ♀	

All injections were given in one cc. of peanut oil, since the amount and type of oil are known to influence the effect of the hormone.[4]

In the carrying out of nasal inspection an assistant entered the animal cages, selected each monkey in turn and held the animal against the mesh work of the cage in such a manner that the face fitted snugly against an opening in the mesh. Thus, with only moderate excitement of the monkeys, 3 observers, who remained outside of the cage, were enabled to examine the animals without knowledge beforehand of the monkey's identity.

* This work was in part supported by funds provided by Doctor George Walker.

1 Mackenzie, J., *Am. Med. Sci.*, 1884, **81**, 365.

2 Fliess, W., *Die Beziehungen zwischen Nase und weiblichen Geschlechtsorganen.* Franz Deuticke, Leipzig und Wien, 1897.

3 Mortimer, H., Wright, R., and Collip, *J. Can. Med. Assn.*, 1936, **35**, 503; 1936, **35**, 615.

† Testosterone acetate and testosterone propionate (Perandren) were furnished through the courtesy of Ciba Company, ketohydroxyoestrin (Theelin) through the courtesy of Parke, Davis and Company.

4 Parkes, A. S., *Lancet*, 1936, **2**, 674.

With a small otoscope study was made of the nasal cavity with reference to the amount of congestion, swelling and secretion. Inspection was also made of the sex skin and genital organs for comparison with nasal changes. Injection was given at completion of the observations. Drawings in color were made by an artist unacquainted with the problem.

Nasal inspection was done on a group of boys and men who were receiving testosterone propionate for various conditions such as cryptorchidism, impotence (absence of penile erection), hypogonadism and atrophic rhinitis. In this group were 5 boys ranging from 18 months to 15 years, a 27, a 29, and a 43-year-old man. Drawings in color were made as in the monkeys.

After a week or more there was seen in the animals receiving massive daily dosages of androgens a marked increase in the congestion, swelling and secretion of the mucosa covering the upper of the 2 conchae present in the macaque. Reddening of the circumgenital skin and development of the genitalia of both males and females[5] was observed, but the onset was not necessarily concomitant with the earliest appearance of nasal phenomena. Increased vaginal secretion was seen in the females but smears revealed neither cornification nor increase in the amount of free vaginal cells.

In monkeys receiving injections but 3 times weekly, nasal changes were not observed until after a longer period, appearing first in animals receiving doses of 4 mg., later in those receiving one mg., and failing to appear within 5 weeks in animals receiving 1/16 mg. or less. It is well to remember that in any quantitative measurement of response the condition of the animal is a significant factor. The monkey quarters used in the experiments are small, so that several shipments of animals were used at different times, hence the monkeys may well have varied in their capacity to respond to the hormones. Nevertheless, there was, beyond doubt, a longer latent period in the appearance of nasal changes when smaller dosages or infrequent administrations were employed.

Histologically, congestion and, in particular, perivascular edema were the most pronounced changes. Although considerable moisture was encountered during nasal inspection of the injected monkeys when they were alive, definite growth changes in the mucous glands were not observed. It may be possible that the fluid observed in the living state was in part associated with the congestion and edema of this region.

As in the monkeys, swelling and congestion were obvious in the

5 Hamilton, J. B., *Anat. Rec.*, 1937, **67** (Supplement), 22.

humans. Some of the patients reported an increased amount of secretion during the period of treatment with a disappearance of secretion after cessation of injections. Upon nasal inspection fluid was observed. Although coryza and other complicating factors are found much more commonly in humans than in monkeys, the nasal mucosa was seen to be definitely affected by adequate dosages of testosterone acetate or testosterone propionate in all 5 boys and the 3 men under observation. Of particular interest is the 29-year-old patient with a condition of atrophic rhinitis of several years' duration. Change in the nasal condition did not occur when control injections were given of oil solution which lacked the androgenic substance, but improvement was obtained following periods of treatment with 6 injections (3 per week), each containing 20 mg. testosterone propionate in one cc. of peanut oil. This improvement lasted from 2 to 4 weeks after a period of administration. Such symptomatic treatment has maintained the nasal mucosa of this patient in a satisfactory condition for the past 9 months. Histological studies have not been done in humans.

A definite relationship between the function of specialized regions of the nasal passages and the presence of the female sex hormone has been conclusively shown by Mortimer and coworkers.[3] The present data demonstrate that male hormone substances also influence the state of this region, both in the female as well as in the male monkey, in boys and in men. Possibly clinical treatment of under-development or hypofunction of this nasal region with hormonal substances may be of use. The 27-year-old man with hypopituitaryism, the immature children and the adult men all exhibited pronounced nasal responses following administration of androgenic substances. At present estrogenic[3] and androgenic substances are being employed in further study of atrophic rhinitis. Caution should be observed, however, in the use of androgens in females, for they inhibit menstruation and also exert a direct masculinizing effect, particularly upon homologues of those organs which in the male respond to male substances.[5] This is especially marked in the development of Skene's ducts into a prostatic type of organ[6] and the clitoris into a penis-like structure.[5]

The narrowing, congestion and secretion of the nasal passageway is significant in regard to infectious processes and also the warming of inspired air. It is well-known that there is normally a sheet of fluid material which is continually passed back by ciliary

[6] Hamilton, J. B., and Wolfe, J. M., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 465.

action to the pharynx, a phenomenon considered significant in preventing lodging of material on nasal areas. It would seem worthwhile to study the effect of gonadal hormones upon the formation and movement of this nasal sheet.

Summary. 1. In 15 monkeys and 8 humans testosterone propionate effected changes in specialized nasal areas. These changes produced by androgens occurred in both male and female monkeys. 2. In monkeys and in humans congestion, swelling and fluid formation were observed grossly. Histologically, perivascular edema was pronounced in the monkeys. 3. The nasal areas affected are similar to those which exhibit vicarious menstruation. Discussion is given as to possible physiological significance of gonad-controlled function of these areas with regard to warming of inspired air, the prevention of infection, and therapeutic use of gonadal substance in conditions of atrophic rhinitis and nasal hyposecretion.

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Effect of Sodium Chloride Therapy on Oestrous Cycle and Hypophysis of Bilaterally Suprarenalectomized Rats.

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Complete suprarenalectomy in rats results in a suppression or irregular appearance of the oestrous cycle.[1, 8] This has been attributed to an intermediate factor, the impaired gonad stimulating function of the hypophysis. Rubin and Krick,[9] and Gaunt,[3, 4] and other investigators have established the efficacy of salt therapy in maintaining the life of adrenalectomized rats. Experiments were conducted in this investigation to determine the influence of sodium chloride administration in restoring (1) gonadotropic function of the hypophysis and (2) the normal oestrous rhythm. Reference to

1 Corey, E. L., and Britton, S. W., *Am. J. Physiol.*, 1934, **107**, 207.

8 Martin, S. J., *Am. J. Physiol.*, 1932, **100**, 180.

9 Rubin, M. I., and Krick, E. T., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 228.

3 Gaunt, R., Tobin, C. E., and Gaunt, J. H., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 134.

4 Gaunt, R., Tobin, C. E., and Gaunt, J. H., *Am. J. Physiol.*, 1935, **111**, 321.

a part of this problem was made by Kutz,[7] who reported that 18 suprarenalectomized rats out of 31 showed normal ovarian cycles on salt therapy.

Vaginal smears of 88 young adult (4-6 months old) albino rats, obtained from 3 different colonies, were examined daily and body weights recorded bi-weekly throughout this study. All animals showed 3-4 normal oestrous cycles of 4-6 days duration, before they were subjected to experimentation. Seventy-four rats were bilaterally adrenalectomized and 14 were kept as controls following a unilateral removal of the adrenal gland.

Of the 74 experimental rats, 28 were given tap water and the remaining 46 received 1 or 2% sodium chloride solution in open dishes in addition to the usual rat diet. Ninety percent of the former group died of typical adrenal insufficiency, the average survival period being 11.6 days (range, 6-19 days). Salt therapy in the latter series of 46 experimental rats was continued for 32 days, discontinued for 3-12 days (average 8 days) until signs of suprarenal insufficiency appeared in all animals, and then resumed again for 23-28 days. This procedure was carried out in order to insure that survival was due only to salt therapy. In the first period of salt administration, 26% died with an average survival period of 16.8 days or 5.2 days longer than rats receiving tap water. The body weight of the remaining 34 animals was slightly above the preoperative level and distinctly below that of the control rats. In the period when salt therapy was discontinued 8 rats died from hypoadrenia despite intraperitoneal injections of normal physiological saline. This is in agreement with Gaunt.[7] During the second period of salt administration, 22 rats survived, showing again slight increases in preoperative weight levels and apparently normal body activity.

The oestrous rhythm, with the exception of one or two cycles, remained essentially normal in 55% of the females during the first

TABLE I.
Bilaterally Adrenalectomized Rats Showing Effects on Oestrous Cycle after Two Periods of NaCl Therapy.

Oestrous Effects	First NaCl Therapy* for 32 days	No NaCl Therapy (3-12 days)	Second NaCl Therapy (23-28 days)
Complete cessation of oestrus	4 rats	17 rats	3 rats
Prolonged or irregular cycles	16 "	10 "	7 "
1-2 cycles prolonged, rest normal	19 "	3 "	8 "
Normal oestrous cycle (4-6 days)	7 "	4 "	4 "

*3 rats successfully mated.

[7] Kutz, R. L., McKeown, T., and Selye, H., Proc. Soc. Exp. Biol. and Med., 1934, **32**, 331.

TABLE II.
Effects on Immature Ovaries of Implanted Hypophyses of Adrenalectomized Rats after Second NaCl Therapy.

Control		Experimental	
Pairs of Rats	Wt. in mg. Ovaries	Pairs of Rats	Wt. in mg. Ovaries
7	12.8	6	12.9
	13.6		11.8
	13.2		13.3
	13.5		12.6
	11.9		13.7
	12.6		12.9
	14.0	Aver.	12.9
Aver.	13.2		
		4	9.7
			9.9
			9.5
			10.1
		Aver.	9.8

and second periods of salt administration (Table I). It was completely inhibited in 9-13%, and prolonged or irregular in 35%. Initial and temporary postoperative oestrous disturbances paralleled the fall in body weight and was probably due to it.[6] The oestrous cycle of the 34 adrenalectomized rats whose salt therapy was discontinued showed essentially the same disturbance as previously noted.[8] During the first course of salt treatment, 3 of the experimental females were successfully mated. They, however, died of typical hypoadrenia when the foetuses were approximately 10-12 days old. The salt administration apparently was inadequate to maintain life during pregnancy.

Of the rats maintained on the second course of salt therapy 10 were sacrificed on the twenty-third day and 10 on the twenty-eighth day, and the gonad stimulating power of their hypophyses assayed by intramuscular implantation of 2 pituitaries into a 22-day female rat. The donors were grouped so that body weights and oestrous effects were essentially similar. The hypophyseal activity was then compared to that of 14 control rats. Table II shows that the gonad-stimulating potency of adrenalectomized rats maintained for 67-72 days on salt therapy is essentially similar to the controls in 60% of the cases. The remaining group showed a decrease of 33% in hypophyseal potency. All the animals of this latter group showed disturbances in oestrus varying from prolonged or irregular cycles to complete cessation.

In view of the hypophyseal-corticoadrenal interrelationship, it

[6] Kroc, R. L., and Martin, S. J., *Am. J. Physiol.*, 1934, **108**, 438.

appears striking to note that normal oestrous activity can be restored at all with salt therapy in the absence of the adrenal glands. Further, the gonad-stimulating power of the hypophysis of adrenalectomized rats remains unimpaired in the majority of instances on salt treatment. Mating was successful in 3 instances. It is possible that adrenalectomized rats showing impairment in ovarian and hypophyseal function may not have ingested adequate sodium chloride. However, these findings further emphasize the secondary importance of the adrenal cortex on gonadal activity. They also confirm the contention that the suprarenal cortex exerts no direct estrogenic effect,[1, 2] since as many rats showed normal oestrus as those that did not.

If the prevalent theory of corticoadrenal function to maintain the normal osmotic balance of the body is correct, perhaps the administration of sodium chloride in adrenalectomized rats serves to restore the normal electrolytic equilibrium of hypophyseal and ovarian tissue in a manner similar to its action on renal tissue.[5]

Conclusion. Sodium chloride therapy in suprarenalectomized rats during our period of observation was effective not only in prolonging life but also in restoring normal hypophyseal-ovarian activity in 55% of the cases.

9575

Cultivation of Rabies Virus.*

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Webster and Clow[1] and Kanazawa[2] reported the first success in cultivating rabies virus. The former accomplished this with a medium consisting of Tyrode solution containing normal monkey serum and minced mouse embryo brain, while the latter propagated the virus in Tyrode solution in the presence of rabbit embryo brain tissue, but without the addition of serum.

2 Fitzhugh, O. G., *Am. J. Physiol.*, 1937, **118**, 677.

5 Ingle, D. J., Wilson, H. W., and Kendall, E. C., *Am. J. Physiol.*, 1937, **118**, 302.

* These studies were supported by the Mary Hooper Somers Fund for Research in Filterable Viruses.

1 Webster, L. T., and Clow, A. D., *Science*, 1936, **84**, 487.

2 Kanazawa, K., *Jap. J. Exp. Med.*, 1936, **14**, 519.

We wish to report our confirmation of the observations of Webster and Clow, since by the procedure which they describe, we have been able to carry the virus through 16 subcultures. Material from the final culture has proved infectious for Swiss mice in dilution of 10^{-2}. In addition a series of passages carried out on the same medium, but with an equivalent amount of normal rabbit serum in place of normal monkey serum yielded results which were similar in all respects to those obtained with normal monkey serum.

Attempts to propagate the virus on media containing a larger ratio of serum to Tyrode and considerably smaller amounts of tissue proved unsuccessful. The fact that Kanazawa propagated the virus in a medium containing rabbit embryo brain, without the addition of serum, suggests that possibly the concentration of serum may have been a factor and that a low concentration of serum or no serum at all, may be more favorable to growth.

Twelve separate attempts were made to propagate the virus on the chorio-allantoic membranes of developing chicks. All yielded entirely negative results. This is in accord with the experience of Waldhecker.[3]

9576 P

Differences in Spread of Dye in Skin of Normal and Tuberculous Guinea Pigs.

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In a study of the skin of guinea pigs, it has been found that the tuberculous animal reacts differently from the normal in regard to the spread of dye which has been injected intradermally.

We have made tests to see if differences could be detected in the spread of dye in the skin around the zone of primary inoculation as contrasted with other areas. The work was started in the course of completing a study of the Koch phenomenon which had been begun by the late Henry Sewall. He had obtained evidence that there are differences in sensitivity in different areas of the skin of the tuberculous guinea pig.

The dye used was pontamine sky blue which we obtained through

[3] Waldhecker, M., *Centralbl. f. Bakt., O.*, 1935, **135**, 259.

the courtesy of Dr. P. D. McMaster, and in general we have followed his technique.[1] We have used the dye as a 2.5%, approximately isotonic solution. The amount of solution injected was 0.025 cc., given with a Dewitt and Herz syringe which has an automatic guard on the plunger, assuring exact dosage. The injections were made under a binocular dissecting microscope under brilliant light, the animal having been anesthetized with sodium amytal.

It was found that there are some differences in the rate of spread of dye, according to varying thickness of the dermis. In the guinea pig the thickness of the dermis in dorsal, lateral, and ventral zones is in the proportion of 2:1.5:1, that is to say, the skin on the back is twice as thick as that on the abdomen. Hence, to be comparable, injections must be restricted to one of these 3 zones. Usually 4 injections, 2 on each side, were made in each animal but in the case of some of the dorsal injections, only 2 were made, each in the mid-line. When the injections were thus limited, the spread in a given animal was practically identical, provided that in the tuberculous animal the indurated border of the primary ulcer was avoided.

In these experiments statistically significant differences have been noted in the spread of the dye between the tuberculous and the normal guinea pig. The dye spreads more slowly in the tuberculous animal. Measurements of the area through which the dye had spread were not significantly different in 1 hour. Every intradermal injection, as Hudack and McMaster[2] have shown, involves a direct injection of dermal lymphatics which the dye enters under pressure and from which it spreads immediately into the subcutaneous plexus. After this phenomenon has been equalized by diffusion, definite differences in spread are observed; thus, after 4 hours, the spread of the dye was nearly 50% greater in the normal than in the tuberculous animals.

In Table I are given the results of one experiment in which the injections were made in the dorsal region in 9 tuberculous and 6 normal guinea pigs. The figures showing significant differences are in bold-faced type. It will be noted in the table that the difference was more accentuated at 24 hours than at 4. This phenomenon becomes still more striking when the injections are made in the ventral zone, because in 24 hours the dye had spread throughout the normal animal but was still restricted to a measurable area in the tuberculous guinea pigs. These experiments have involved 80 injections in 13 tuberculous and 46 in 7 normal guinea pigs with consistent results.

[1] McMaster, P. D., *J. Exp. Med.*, 1937, **65**, 347.

[2] Hudack, S. S., and McMaster, P. D., *J. Exp. Med.*, 1933, **57**, 751.

TABLE I.
Measurement of Mean Area of Spread of Dye Injected into the Dorsal Region in Guinea Pigs.

	1 hr.		4 hr.		24 hr.	
	Mean sq. mm.	Standard deviation	Mean sq. mm.	Standard deviation	Mean sq. mm.	Standard deviation
Normal 18 injections 6 animals	121 ± 5.6	35.6	**237** ± 11.4	71.7	**678** ± 40.4	253.9
Tuberculous 18 injections 9 animals	103 ± 4.7	29.4	**166** ± 10.1	63.6	**411** ± 21.5	135.5

No significant differences were found in the spread of the dye in the tuberculous guinea pigs in respect to the degree of the tuberculin reaction, for the spread was about the same in animals which reacted from + to ++++. However, tuberculous guinea pigs in the terminal stages of the disease, when they are losing weight and have become negative to the tuberculin test, have lost the power of restricting the spread of the dye, which then diffuses as rapidly as in the normal guinea pig.

An increased power to restrict diffusion of dye does not seem to characterize the tuberculous state in the rabbit, for in a group of 3 normal and 3 tuberculous rabbits, intradermal injections of the dye spread at the same rate.

These observations show that there is a normal difference in the rate of diffusion of dye in proportion to the thickness of the dermis. This rate of diffusion, as indicated by injections of dye, becomes altered, that is, delayed, in the tuberculous guinea pig. It may be possible that this phenomenon is one factor among the complicated processes involved in a tuberculin skin test. Thus, if we may assume that the rate of diffusion of dye is approximately an indicator of the diffusion of the protein, an animal in which the tuberculous infection has induced a delay in the rate of diffusion in the tissues is able to react to a smaller amount of tuberculo-protein, because a larger proportion of the material is retained in contact with the cells instead of being rapidly diffused throughout the body. Thus the rate of diffusion affects the concentration of material at the point of injection. The converse of this phenomenon is illustrated in the observations of Duran-Reynals[3] and of Thomas and Duran-Reynals,[4] in which they showed that an animal positive to a given amount of tuberculo-protein can be rendered less reactive by the introduction of a spreading factor with the protein.

3 Duran-Reynals, F., *J. Exp. Med.*, 1933, **58**, 451.

4 Thomas, R. M., and Duran-Reynals, F., *J. Exp. Med.*, 1935, **62**, 39.

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Serum Phosphatase Activity in Generalized Osteosclerosis Due to Chronic Fluorine Intoxication in Man.

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Flemming Møller and Gudjonsson[1] called attention to a peculiar form of generalized osteosclerosis, hitherto unknown, occurring in Danish workers exposed to dust of cryolite, a fluorine compound (Na_3AlF_6). The condition is characterized[1-5] by a diffuse increase in density of the bones, particularly of the spine, pelvis and ribs, and by calcification of ligaments and tendinous muscle attachments. Because of marked periosteal proliferation and the calcification of contiguous fibrous tissues, the sclerotic bones are often irregular in contour. There may be considerable impingement also upon the medullary cavity. The spine and thoracic cage ultimately become rigid, resulting in loss of mobility and vague "rheumatic" pains; but clinical signs and symptoms are otherwise remarkably few, even in advanced cases. At necropsy,[2, 6] the sclerosed bones are found to be heavy (up to 3 times the normal weight) with rough surface and relatively brittle texture. Histologically, the peculiar if not pathognomonic osseous structure is characterized by the partial deposition of lime salts in the form of irregular granules. The fluorine content of the bone ash[2, 5] is increased to about 10 times the normal values.

While distinctly less common than abnormalities of dentition, ("mottled enamel"),[19] skeletal changes due to chronic fluorine intoxication probably occur more widely in man than is generally appreciated. A number of cases of osteosclerosis have been reported from North Africa[7] and from India.[8] In the United States, Bishop[5] recently described typical skeletal changes in a man who for 18

1 Møller, P. F., and Gudjonsson, S. V., *Acta radiol.*, 1932, **13**, 269.

2 Roholm, K., *Fluorine Intoxication*, Lewis & Co., London, 1937.

3 Roholm, K., *Arch. F. Gewerbepath.*, 1936, **7**, 255.

4 Roholm, K., *J. Ind. Hygiene*, 1937, **19**, 126.

5 Bishop, P. A., *Am. J. Roent.*, 1936, **35**, 577.

6 Bauer, J. T., Bishop, P. A., and Wolff, W. A., *Bull. Ayer Clin. Lab.*, 1937, **3**, 67.

19 Smith, M. C., Lantz, E. M., and Smith, H. V., *J. Am. Dent. Assn.*, 1935, **22**, 817.

7 Spéder, E., *Bull. Mém. Soc. Radiol. Méd. France*, 1936, **24**, 200.

8 Shortt, H. E., Pandit, C. G., and Raghavachari, T. N. S., *Indian Med. Gaz.*, 1937, **72**, 396.

years had handled finely ground rock phosphate (a complex calcium-fluorphosphate) in a "superphosphate" fertilizer factory.

In the literature on chronic fluorine intoxication in man, no values could be found for serum phosphatase, an enzyme to which is ascribed an important rôle in bone formation. Marked increases in serum phosphatase activity are known to occur in other diseases presenting widespread osteosclerosis, notably in Paget's disease,[9] carcinoma with osteoplastic metastases,[9] and in some cases of Albers-Schönberg disease.[10] In chronic fluorine intoxication produced experimentally in animals, Phillips[11] reported mean plasma phosphatase values approximately twice those of control animals whereas Smith and Lantz[12] found no difference between their treated and control groups and De Eds[13] obtained inconclusive results.

We have determined the phosphatase activity of the serum in 20 cases of chronic fluorine intoxication (Table I) representing various stages of bone involvement, from advanced osteosclerosis to roentgenographically negative cases. The subjects, 16 men and 4 women employed 8-34 years in the Copenhagen cryolite factory, were selected from the group originally studied roentgenographically by Flemming Møller and Gudjonsson[1] and later *in extenso* by Roholm.[2] Blood samples were obtained 2-3 hours after a light breakfast. Serum phosphatase activity was determined by the Bodansky method within a few hours after collection, inorganic phosphorus by the Kuttner-Lichtenstein method, calcium by the Clark-Collip modification of the Kramer-Tisdall method.

Despite the presence of extensive osteosclerosis in many subjects, the serum phosphatase activity was found to be within the normal range (1.0-4.0 Bodansky units per 100 cc. serum) in 14 of 20 cases. In 6 instances the serum phosphatase activity was slightly increased, 7.0 Bodansky units being the highest value observed. There was no proportionality between the level of serum phosphatase activity and the degree of osteosclerosis. The inorganic phosphorus values were within normal limits and the serum calcium, in confirmation of Roholm[2] and Bishop,[5] was normal or slightly increased.

The absence of appreciable increases in serum phosphatase activity in our cases may, of course, be due simply to the extremely slow development of osteosclerosis. The average period of exposure to

9 Gutman, A. B., Tyson, T. L., and Gutman, E. B., *Arch. Int. Med.*, 1936, **57**, 379.

10 Bodansky, A., and Jaffe, H. L., *Arch. Int. Med.*, 1934, **54**, 88.

11 Phillips, P. H., *Science*, 1932, **76**, 239.

12 Smith, M. C., and Lantz, E. M., *J. Biol. Chem.*, 1935, **112**, 303.

13 DeEds, F., *J. Am. Dent. Assn.*, 1936, **23**, 568.

TABLE I.
Serum Phosphatase Activity, Inorganic Phosphorus and Calcium in 20 Cases of Chronic Fluorine Intoxication in Man.

No.*	Sex	Age	Years of exposure	Serum		
				Phosphatase (Bodansky units per 100 cc.)	Inorg. P (mg. %)	Calcium (mg. %)
Group without osteosclerosis roentgenographically.						
17	M	37	12	3.5	2.7	—
39	M	31	9	4.1	3.1	—
43	M	33	8	1.8	3.4	10.1
Group with osteosclerosis of 1st phase.†						
52	F	58	22	3.3	3.1	9.9
4	M	38	11	3.3	2.8	11.3
30	M	41	8	4.4	3.2	—
45	M	35	14	1.8	2.7	—
62	F	59	22	3.3	3.4	11.2
65	M	45	13	3.3	2.8	11.2
Group with osteosclerosis of 2nd phase.†						
2	M	42	15	2.8	2.6	12.0
6	M	38	14	7.0	2.6	11.4
19	M	35	13	2.6	2.7	—
20	M	38	18	1.6	2.8	—
23	F	31	9	2.7	4.0	—
32	M	33	9	6.2	2.9	11.1
41	F	61	34	5.3	3.4	10.5
50	M	59	14	3.8	2.8	—
Group with osteosclerosis of 3rd phase.†						
15	M	62	21	2.7	3.2	10.9
34	M	48	18	4.3	2.8	11.6
51	M	66	15	3.6	3.6	11.2

*The numbers correspond with those in Roholm's monograph,[2], which see for further details regarding these subjects.

†1st phase: just recognizable roentgenographic changes, slightly increased density. 3rd phase: marble-like density, pronounced periosteal proliferation and calcification of ligaments.

fluorine for the group with early bone changes (1st phase) was 9.3 years, for the group with advanced osteosclerosis (3rd phase) 21.1 years.[2] This is probably not the only factor involved, however, since Paget's disease is often equally slow in development, yet the serum phosphatase activity may exceed 100 Bodansky units per 100 cc. A likely further possibility is that the mechanism of bone formation in chronic fluorine intoxication differs in some significant though as yet obscure manner from that operating in other osteosclerotic conditions. This difference in mechanism of bone formation is suggested also by the unusually pronounced periosteal activity, by the presence of granules, and by the few active osteoblasts and the narrow osteoid borders seen in bone sections.

We think it improbable that the normal serum phosphatase activity found in most of our cases is due to direct inhibition of serum phos-

phatase by fluorides. When serum of a case of chronic fluorine intoxication was mixed with a very active blood sample obtained from a case of metastatic osteoplastic carcinoma (64.6 Bodansky units per 100 cc.) the resulting serum phosphatase activity was not lower than calculated. Fluoride in concentrations as high as 0.01 molar does not significantly inhibit the phosphatase activity of normal or Paget serum[14] *in vitro*. "Alkaline" bone phosphatase activity is similarly not appreciably inhibited by fluorides *in vitro*, according to most investigators.[12, 15, 16, 17] Fluorides do inhibit "acid" phosphatases, which may play a rôle in bone formation,[15, 17, 18] but how this affects the level of "alkaline" serum phosphatase activity is not now clear.

A practical point worth emphasizing is that chronic fluorine intoxication should be considered as a possible cause of obscure generalized osteosclerosis, particularly if associated with normal serum phosphatase activity.

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Excretion of Inulin, Creatinine, Xylose and Urea in the Sheep.

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We are presenting a summary of observations on the renal excretion of inulin, creatinine, xylose, and urea in a single normal sheep. These observations were made in 1934 and since the results were concordant with similar observations on the dog,[1] further investiga-

TABLE I.
Summary of Observations on Sheep (Weight, 30.0 kg.).

	Experiments	No. of Observations	Mean
Inulin clearance	3	10	58.5 cc./min.
Creatinine/inulin clearance ratio	3	10	1.03
Xylose/inulin clearance ratio	1	2	.73
Urea/inulin clearance ratio	2	6	.52

14 Gutman, A. B., and Gutman, E. B., unpublished data.

15 Robison, R., and Rosenheim, A. H., *Biochem. J.*, 1934, **28**, 684.

16 Folley, S. J., and Kay, H. D., *Erg. Enzymforsch.*, 1936, **5**, 159.

17 Gutman, E. B., Sproul, E. E., and Gutman, A. B., *Am. J. Cancer*, 1936, **28**, 485. For contrary opinion, see 13 and 16.

18 Phillips, P. H., and Hart, E. B., *J. Biol. Chem.*, 1935, **109**, 657.

1 Shannon, James A., *Am. J. Physiol.*, 1935, **112**, 405.

tion seemed unnecessary. The observations were made on an unanesthetized animal, restrained upon an animal board. The experimental procedure and chemical methods were similar to those used in observations on dogs.[1] The urine flow at which these observations were made varied from 0.82 to 2.20 cc. per minute.

9579 P

Application of Vital Dyes to the Study of Sheath Cell Origin.

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That the spinal ganglion cells and sheath cells of Schwann have a common origin in the neural crest, his been accepted generally since Harrison's original experiments in 1904.[1] When the dorsal portion of spinal cords of anuran embryos were removed (elimination of the ganglionic crest), the larvae lacked spinal ganglia and sensory nerves, and the motor nerves present were devoid of sheath cells. Recently Raven[2, 3] employing a different experimental approach assigns a cord origin to the sheath cells and claims that Harrison's conclusions were not justified, since in eliminating the neural crest he also destroyed the dorsal part of the spinal cord, thus not only removing such presumptive sheath cells as may originate there, but obstructing by deformation of the cord the dorsal migration of these elements which are supposed to lie in the ventral portion of this structure. Raven also concludes from his experimental results that sympathetic elements arise from both neural crest cells and from the ventral portion of the spinal cord. His findings do not support those of Müller and Ingvar[4, 5] and Van Campenhout[6] who claim a neural crest origin exclusively for sympathetic ganglion cells, but they do support in part those of Kuntz and Batson,[7] and Kuntz,[8]

1 Harrison, R. G., *Sitz. Ber. Niederrh. Ges. Natur. u. Heilkunde*, 1904, Bonn (*v.* also Harrison, R. G., *J. Comp. Neur.*, 1924, **37**, 123).

2 Raven, Chr. P., *Arch. f. Entw.-mech.*, 1936, **134**, 122.

3 Raven, Chr. P., *J. Comp. Neur.*, 1937, **67**, 221.

4 Müller, E., and Ingvar, S., *Upsala Läkaförenings förhandlingar Ny följd*, 1921, **26**.

5 Müller, E., and Ingvar, S., *Arch. f. mikr. Anat. u. Entw.-mech.*, 1923, **99**, 650.

6 Van Campenhout, E., *J. Exp. Zool.*, 1930, **56**, 295.

7 Kuntz, A., and Batson, O. V., *J. Comp. Neur.*, 1920, **32**, 335.

8 Kuntz, A., *J. Comp. Neur.*, 1922, **34**, 1.

and Jones,[9] who assign a neural tube origin for the sympathetic elements.

Raven interchanged trunk neural crest and also median part of medullary plate (future ventral portion of spinal cord) between embryos of *Amblystoma mexicanum* (axolotl) and *Triton taeniatus* (xenoplastic grafts). Axolotl (donor) cells in Triton embryos could be recognized by their larger nuclear sizes. Employing variation curves for nuclear size difference in both donor and host cells, Raven employed statistical methods in the analysis of his results. Although he obtained evidence for a dual origin of sympathetic ganglion cells, he says that the sheath cells of Schwann are derived exclusively from the tube. These cells are regarded as originating chiefly from the ventral portion of the cord, and he assumes that they migrate out either along the dorsal or the ventral roots of the spinal nerves.

The problem has been further investigated by the author using vital dyes in the following manner, (1) the median portion of the medullary plate of Amblystoma embryos (stages 14-15) was stained with Nile blue sulphate, (2) the corresponding trunk neural crest was likewise stained, and (3) the median portion of the plate was stained with neutral red, and the corresponding crest was stained with Nile blue. The stain was applied to the desired region of the embryo by means of appropriate sized pieces of cellophane previously impregnated with the dye. In several hours the dye was transferred from the cellophane carrier to the embryo, resulting in a brilliant blue or red stain respectively. The embryos were kept to stages 35-40 when they were fixed according to the method of Stone[10] for the preservations of the dyes.

Microscopic examination of such vitally stained embryos gave no evidence that the early sheath cells originate from the ventral part of the spinal cord. When this portion of the cord was stained blue, the cells of the spinal ganglia and the sheath cells possessed the natural brownish yellow pigment characteristic of the unstained neural cells.

In staining the neural crest, it has so far been impossible to obtain any embryos without some diffusion of the dye into the very dorsal part of the cord. Whereas the spinal ganglion cells and the sheath cells in such cases were correspondingly blue, the experiments so far have not been sufficiently conclusive to say whether

[9] Jones, D. S., *Abstract. Wistar Institute Biobliographic Service*, No. 297, October 15, 1937.

[10] Stone, L. S., *Anat. Rec.*, 1932, **51**, 267.

the colored sheath cells take origin from the stained neural crest as do the ganglia or whether they originate from the dorsal part of the definitive cord *per se,* which also possessed some blue stain. If the sheath cells take origin from the ventral portion of the cord and migrate out by way of the ventral roots, then sheath cells possessing the natural unstained brownish pigment should be seen. Their absence in such preparations is evidence that they do not originate from this source in early embryos. That they could not have migrated out by way of the dorsal roots is also obvious from the fact that the embryos were studied prior to the development of sensory roots.

In spite of the want of definite proof there are some indications of a common origin (neural crest) for spinal ganglion cells and the early sheath cells. This is based on evidence of a lateral and ventral migration of stained crest cells across the mid-dorsal line indicating that spinal ganglion cells and the sheath cells on one side may have their origin, at least in part, from the contralateral stained crest. It is hoped that experiments which are in progress will yield more decisive results. The evidence so far obtained by this method does not lend any support to the view that the early migrating sheath cells take origin from the ventral or lateral portions of the spinal cord. Further, it does not disprove that the sheath cells in later stages may come from this source.

9580

Virus Isolated from Nasal Washings during Acute Poliomyelitis in New York City in 1935.*

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The virus of poliomyelitis has been infrequently detected in the nasal secretions of patients during and following an attack of poliomyelitis. We have reviewed the literature and considered that until the summer of 1935 when this study was begun, the virus had been isolated from human nasopharyngeal secretions only 11 times.[1-7]

* This research was aided by grants from the Scientific Committee of the President's Birthday Ball Fund and the Rockefeller Foundation.

[1] Kling, C., Pettersson, A., and Wernstedt, W., *Communications de l'Institut Medical de l'État a Stockholm,* Tome III, 1912.

The criteria which we used to determine the presence of virus were: (a) the transmission of the clinical disease to monkeys, *viz.*, fever, tremor, ataxia and paralysis, (b) the typical histopathological changes in the spinal cord and medulla, *viz.*, perivascular and interstitial infiltration and neuronophagia, particularly of the gray matter in the anterior horns, and (c) the serial passage of the disease to other monkeys. The above requisites were present in all isolations except those of Kling[1] in which 2 of the 3 requirements were met. Since then, Kramer[8] has recorded the isolation of the virus from 2 additional cases. Our report describes a fourteenth instance (Table I.)

TABLE I.
Summary of Isolations of Virus from Human Nasopharyngeal Secretions in Poliomyelitis.

Investigators	Type of Case	Day of Illness Virus Isolated
Kling and co-workers[1]	Case 10 paralytic	4
	" 16 "	4
	" 22 "	4
Dubois and co-workers[2]	Non-paralytic	17
Taylor and Amoss[3]	Abortive	4
	Paralytic	5 days before onset of paralysis
Lucas and Osgood[4]	"	4 months after onset of second attack
Levaditi and Willemin[5]	"	8
Paul and Trask[6]	Abortive	1
	"	1
Paul and co-workers[7]	"	1
Kramer and co-workers[8]	Paralytic	16
	"	13
Stillerman and Brodie	"	9

An opportunity to test nasopharyngeal secretions for the virus presented itself at the Willard Parker Hospital during the 1935 New York City poliomyelitis outbreak. With a view of determining how long the virus survived in the nasopharynx, serial nasal washings from 15 patients, 9 paralytics and 6 non-paralytics, were

2 Dubois, P. L., Neal, J. B., and Zingher, A., *J. A. M. A.*, 1914, **62**, 19.

3 Taylor, E., and Amoss, H. L., *J. Exp. Med.*, 1917, **26**, 745.

4 Lucas, W. P., and Osgood, R. B., *J. A. M. A.*, 1930, **60**, 1611.

5 Levaditi, C., and Willemin, L., *Ann. Inst. Past.*, 1931, **46**, 233.

6 Paul, J. R., and Trask, J. D., *J. Exp. Med.*, 1932, **56**, 319.

7 Paul, J. R., Trask, J. D., and Webster, L. T., *J. Exp. Med.*, 1935, **62**, 245.

8 Kramer, S. D., Sobel, A. E., Grossman, L. H., and Hoskwith, B., *J. Exp. Med.*, 1936, **64**, 173.

tested. The washings were obtained at weekly intervals up to the 3rd, and in some cases during the 4th and 5th weeks of the disease. Although several nasal washings were tested from each of these 15 patients, the virus was demonstrated in only one specimen.

The nasal washings were obtained with the patient lying on one side or in the sitting position with the head lowered. A soft rubber bulb syringe was inserted into one nostril and the superior portion of the nasal passage irrigated with 60-75 cc. of sterile distilled water or saline. The drippings were caught in a basin and then used to flush the other nostril. This was repeated several times. The washings were kept frozen until filtered. A Seitz filter with a single pad was used. The filtrate was concentrated *in vacuo* at 35°-38°C. until the volume was reduced to 2-4 cc. This required 3-5 hours. The sterility of the filtrate was tested by culture and by inoculation into mice.

Each concentrated filtrate was inoculated into a *Macacus rhesus* monkey, 1 cc. intracerebrally and the remainder intraperitoneally. These animals were examined daily and the temperature recorded. Whenever symptoms and signs indicative of poliomyelitis were manifest, the animal was sacrificed for histopathological study and animal passage.

Poliomyelitis virus was recovered from only one patient. This was a 9-year-old girl, who for one week had had a cold which did not improve. On October 5, 1935, she developed headache, vomiting, and fever. The following day when she was admitted to the hospital, her temperature was 103.2°F. and a weakness of the right side of her face was found, which persisted until her discharge from the hospital 19 days later. Blood-tinged spinal fluid (traumatic) was obtained. A diagnosis of bulbar poliomyelitis was made. The nasal passages were irrigated 4, 9, 15, and 20 days, and 5 weeks after the onset of her illness.

The specimen obtained from this bulbar case on the 9th day of illness, produced poliomyelitis in a monkey. The animal developed the typical experimental disease with flaccid paralysis which progressed to a complete quadriplegia 8 days after inoculation. An emulsion made from the spinal cord of this monkey, produced paralysis on the 6th day in a second animal. In the 3rd passage, 10 of 11 monkeys developed extensive paralysis in from 6 to 14 days. The histological sections of the first passage were lost but those obtained from monkeys of the 2nd and 3rd passages revealed the characteristic lesions of experimental poliomyelitis.

The virus isolated from this patient appeared to be highly viru-

lent, for 1 cc. of a 5% suspension of the spinal cord obtained in the second passage, diluted 200 times, produced infection. Neutralization tests with this virus and a passage virus (FL strain) were done on sera obtained from patients during the acute and convalescent stages of the disease in 1935. In these experiments, both strains of virus reacted similarly to 25 sera, 6 neutralizing and 19 failing to neutralize each strain.[9] These results differed from those of Howitt[10] and Paul and Trask,[11] who found that human convalescent sera more often neutralized a recently isolated human strain than a passage strain. The above mentioned investigators may have obtained neutralization more often against their recently isolated strains because they were less virulent than the passage strains. An attempt to reinfect 6 monkeys having residual paralysis caused by the FL strain, using our 1935 strain, resulted in one definite and one questionable reinfection.[9]

The virus was not isolated from any nasal washing other than that obtained on the 9th day of illness from this patient. Whether it was absent in the other specimens, or was inactivated by the natural neutralizing property of nasal secretions,[12] or was not demonstrated because of the technical difficulties inherent in the method employed, is difficult to state. Some of the virus might have been lost during the dilution of the nasal secretions by irrigation, and the sterilization of the nasal washings by filtration. The use of a single monkey for testing each specimen may be inadequate. It is interesting to note that the virus has never been isolated more than once from the same case of poliomyelitis by any investigator.† Flexner and Amoss[13] demonstrated the presence of the virus in tonsils and pharyngeal tissue of 5 of 10 human beings that died of poliomyelitis during the first 7 days of their infection, but were unable to detect it in 4 fatal cases at later periods of the disease.

Washings obtained from 2 paralytic cases on the 4th day of illness, and a non-paralytic on the 2nd day, were followed by what appeared to be experimental poliomyelitis with flaccid paralysis. However, in neither instance was the diagnosis substantiated by histo-pathological examination or monkey passage.

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9 Brodie, M., Fischer, A. E., and Stillerman, M., *J. Clin. Invest.*, 1937, **16**, 447.

10 Howitt, B., *J. Infect. Dis.*, 1933, **53**, 145.

11 Paul, J. R., and Trask, J. D., *J. Exp. Med.*, 1933, **58**, 513.

12 Amoss, H. L., and Taylor, E., *J. Exp. Med.*, 1917, **25**, 507.

† The one exception is Kling and co-workers,[1] whose findings were inconclusive because the pathological changes they describe in the central nervous system of inoculated monkeys do not appear to be characteristic of poliomyelitis.

13 Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1919, **29**, 379.

Conclusion. Poliomyelitis virus was isolated from the nasopharyngeal secretions of a bulbar case on the 9th day of illness.

The authors wish to acknowledge their appreciation to Dr. Morris Schaeffer of the Bureau of Laboratories, New York City Department of Health, for his valuable suggestions during this work.

9581 P

The Culture and Division Rate of *Dileptus gigas.*

S. RUDIN. (Introduced by J. A. Dawson.)

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Although pedigree isolation cultures of protozoa have been carried on during the last 50 years, relatively few investigators have experimented with carnivorous forms. Woodruff and Spencer[1] and Woodruff and Moore[2] kept *Spathidium spathula* alive for long periods without degeneration, conjugation or endomixis on a diet of *Colpidium colpoda.* Beers,[3] feeding *Didinium nasutum* on *Paramecium caudatum* succeeded in attaining 1384 generations in about a year's time, without any degeneration or internal reorganization of any kind. With these results in mind it was decided to attempt to find a suitable diet for the carnivorous ciliate, *Dileptus gigas,* and to ascertain its division rate on such diet in isolation pedigree cultures.

In 1936 a number of individuals of *Dileptus gigas* were isolated from some of the writer's stock cultures. These cultures were originally collected from Van Cortlandt Park Pond, New York. By preliminary feeding experiments it was soon ascertained that the large, blue *Stentor coeruleus* made an excellent food basis for *Dileptus* and this readily cultured organism[4] was therefore selected as the standard food supply for the experiment. Visscher[5] has described the interesting and unusual manner in which *Dileptus* manages to attack, paralyze, and cytolyze by means of the powerful trichocysts (toxicysts), stentors much larger than itself and feast upon its prey. It is interesting to add that *Stentor,* itself predominantly car-

1 Woodruff, L. L., and Spencer, H., *J. Exp. Zool.,* 1924, **39**, 133.

2 Woodruff, L. L., and Moore, E. C., *Proc. Nat. Acad. Sci.,* 1924, **10**, 183.

3 Beers, C. D., *Am. Nat.,* 1929, **43**, 125.

4 Gerstein, J., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 210.

5 Visscher, J. P., *Biol. Bull.,* 1923, **45**.

nivorous, was grown on *Blepharisma undulans,*[4] the latter being supplied with a mixed diet.

On Nov. 21, 1936, two series, consisting of 4 pure lines each, were established. All of these 8 lines were the progeny of a single *Dileptus* isolated from stock culture on Nov. 18. The organisms were kept in Maximow culture dishes in a moist chamber and were isolated daily (occasionally every other day) by capillary pipette under a binocular dissecting microscope. Fresh medium (spring water) and a sufficient number of healthy, washed stentors to insure an excess of food (approximately 25) were supplied at each isolation. The total amount of culture medium used in each dish was 1.5 cc.

Control cultures were set up at the beginning of the experiment. The medium used in these was that from which the stentors were taken and contained only such bacteria as were present there. Survival periods for *Dilepti* placed in the stentor-free medium varied from one to 20 days. Occasionally a *Dileptus,* if well-fed before being placed in this medium, would divide once but never more. It is thus clear that the continued growth and division of *Dileptus* in these experiments are dependent solely upon the presence of *Stentor* in the culture medium.

An attempt was made to keep environmental conditions as constant as possible. The greatest variable encountered was the temperature, which ranged from less than 15°C. to almost 30°C. throughout the course of the experiment. This factor is responsible for most of the deviations shown in the division rate graph for *Dileptus.*

The organisms have been successfully cultivated in isolation culture for a period of 220 days (Nov. 21, 1936, to June 24, 1937), during which time 213 generations have been attained. Conjugation was prevented by the daily isolations. Encystment occurred in one case when the temperature was approximately 13°C. but the encysted *Dileptus* was discarded and replaced with a reserve pedigree individual of the same generation. Occasionally an individual died, but every case of death was accounted for by some accident or imperfect technique.

The graph (Fig. 1) which represents an average of all lines of both series, shows the daily division rate, averaged for 5-day periods. No evidence of decline in division rate is shown. Explanations of high and low points in division rate are given in the explanation of the figure.

Within the present limits of the experiment there is no evidence

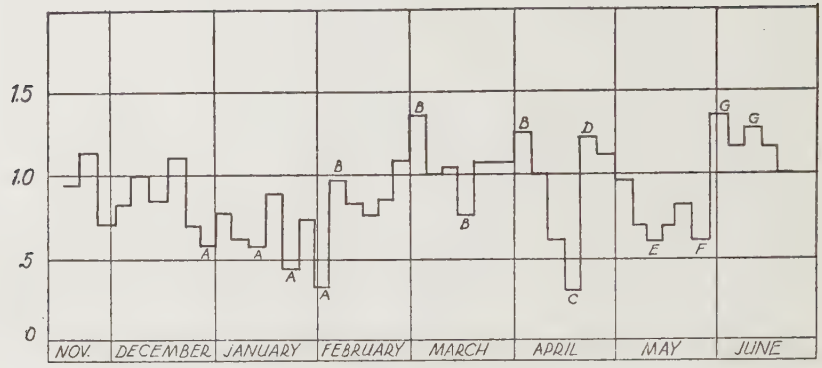

FIG. 1.

The Culture and Division Rate of *Dileptus gigas.*

A—Low division rate due to low temperature, average 15° C during periods indicated.
B—Variations due to fluctuations in temperature of artificial heating device.
C—Low division rate due to accidental exposure of culture dishes to direct sunlight.
D—High division rate due to removal of organisms to warm shaded place. Temperature average, 25° C.
D-E—Gradual drop of average temperature from 25° to 21° C.
F—Low division rate due to change of technicians during illness of author.
G—Temperature average, 28.5° C.

to indicate any "life cycle" or morphological or physiological degeneration of the experimental animals. This work thus corroborates that of earlier investigators[1-4] who used similar culture methods. More extensive work is now in progress.

The author wishes to acknowledge his great indebtedness to Dr. J. A. Dawson, without whose constant supervision, suggestions, and criticisms, this work would have been impossible.

9582 P

Assay of Thyrotropic Hormone on Day-Old Chicks.

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Of the several methods employed in assays of thyrotropic hormone that utilizing the structure and weight responses of the thyroids of guinea pigs is the most commonly used.[1, 2] Day-old white leghorn chicks have been found not only to respond more sensitively than guinea pigs but also to give a relatively greater weight increase and to exhibit a more uniform thyroid structure.

1 Aron, M., *C. R. Soc. de Biol.*, 1929, **102**, 682.

2 Rowlands, I. W., and Parkes, A. S., *Biochem. J.*, 1934, **28**, 1829.

The preparation used was a partially purified alkaline extract of beef anterior pituitary.[3] Although a response is elicited by a single dose in 24 hours, 5 daily injections were found to give a maximal reaction. Further division of doses into 2 a day for 5 days gave a somewhat greater response.

One hundred and seventy-five injected chicks and 36 uninjected controls furnish the basis for the assay curve. Each sample of extract was tested on 5 chicks. At autopsy the thyroids, dissected under a binocular microscope, were weighed and body weight and sex recorded. The total dose was divided into 5 daily subcutaneous injections. It was found advisable to do the autopsy 24 hours after the last injection. Table I gives a representative series of the mean thyroid weights and total dose of extract used. Most dosages were repeated on different hatchings, with separate batches of extract. Total doses varied from 0.1 to 20.0 mg. Increases in thyroid weight were produced by increased dosages up to 10.0 mg. but beyond this there was no further increase in thyroid weight. Thus, a maximum thyroid weight is produced with 100 times the minimum stimulating dose. The larger doses produced thyroids averaging 6 to 7 times the mean weights of controls.

TABLE I.
Effect of Thyrotropic Hormone Injection on Thyroid Weight of Guinea Pigs and Day-Old Chicks.

Total Dose in mg.	Chicks 6 Days of Age			Guinea pigs Body Wt. 150-200 gm.		
	No. of Animals	Mean Thyroid Wt., mg.	Standard Error of Mean	No. of Animals	Mean Thyroid Wt., mg.	Standard Error of Mean
Controls	6	2.8	±0.5	5	22.8	±6.6
0.1	4	3.9	±0.7	—	—	—
0.4	5	5.5	±1.2	5	20.2	±3.8
1.0	—	—	—	6	31.0	±6.1
1.2	5	6.3	±0.9	—	—	—
2.0	5	7.8	±1.2	6	41.5	±6.0
4.0	5	10.0	±1.8	5	38.8	±3.2
8.0	4	14.9	±2.9	4	60.4	±7.6
10.0	5	20.1	±6.6	—	—	—
16.0	—	—	—	5	63.9	±14.8
20.0	5	20.3	±9.1	—	—	—

Thyroids of chicks receiving smaller amounts showed histological evidence of activity. The high degree of activity found in the 0.1 mg. group indicates that even lower doses are detectable histologically.

Several specimens of blood serum, urine extracts, and an extract

[3] Smelser, G. K., *Am. J. Ophthalmology*, 1937, in press.

of liver, prepared in the same manner as the pituitary substance, were ineffective, demonstrating the specificity of the chick thyroid response.

For comparison with the chick, 65 guinea pigs in groups of 5 each were injected with the same thyrotropic preparation in doses varying from 0.1 to 16.0 mg., 5 daily subcutaneous injections being given and autopsies performed 24 hours after the last injection. All animals were from the same colony and weighed from 150 to 200 gm. Table I shows that definite thyroid weight increase is not attained with less than 1.0 mg. of extract and further increase did not occur with doses above 8.0 mg. The thyroid weight increase thus reached a maximum with but 8 times the minimal stimulating dose. The larger doses produced thyroids averaging only about 3 times the mean weight of the controls. Doses too small to give a weight increase produced activation structurally, but such a dose (0.4 mg.) was at least some 4 times the amount necessary to give a comparable stimulation of chick thyroids. Normal guinea pig thyroids are more variable in weight and structure than those of baby chicks. In the latter there is not the difference in the size and morphology between the peripheral and the centrally placed acini which is characteristic of the guinea pig. Additional advantages are that the temperature of the environment is easily controlled and feeding is simple and inexpensive. An unlimited supply of chicks of standard stock, weight, and age is always available at a cost of about one-fifth that of the guinea pig.

Comparison of thyroid weights, obtained in testing unknown preparations, with values in a standard curve, yields only an approximate measure of the potency when 5 chicks are employed. However, when comparing normal and slightly stimulated glands, less variation occurs, and groups of 5 may be used. The chick thyroid weight test is not intended to supersede histological methods entirely, a combination of the two techniques being advisable. When marked thyroid hypertrophy is obtained, histological investigation may be omitted, but when questionable stimulation or none at all occurs the glands should be studied histologically.

9583 P

Bacteriology of the Uterus with Special Reference to Estrogenic Hormones and the Problem of Pyometra.*

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Mice and rabbits receiving large doses of estrogens develop pyometra.[1-6] Estrogenic hormones may induce physiological changes favoring a leucocytic invasion of genital tissues.[2, 4] It has been suggested that this inflammatory condition may be related to the leucocytic infiltration occurring during normal post-estrus. We have tried to determine whether bacteria are present in the uteri of normal mice and whether estrogenic hormones induce changes resulting in bacterial invasion of the uterus.

Mice from several strains and of varying ages were used. All that were treated with estrogens (hydroxy-estrin benzoate) were young, sexually mature, virgin females. The estrogen, in oily solution, was injected subcutaneously, usually in a single dose of 500 I.U. (1/20 cc.). The mice were killed after intervals of 2, 4, 7, 10, 14, and 15 days following the initial injection (Table II). Bacteriological studies were made on the uteri of immature, mature virgin, multiparous and castrated females; of mice receiving injections of oil alone and of the blood of injected and untreated mice (Table I). In a third series one uterine horn was cut from the cervix and

TABLE I.
Occurrence of Bacteria in Uteri or Blood of Mice Used as Controls.

Type of control	No. of mice	Age or treatment	No. with bacteria	No. without bacteria
Immature	5	30 days	0	5
Virgins	18	33 to 157	1	17
Castrated	7	Adult	0	7
Multiparous	11		1	10
Virgins	4	Oil injected	0	4
Blood bact.	7	No injections	0	7
	6	500 i.u.	0	6
	6	500 i.u. × 2	0	6

* This investigation has been supported in part by the Anna Fuller Fund.

1 Burrows, H., and Kennaway, N. M., *Am. J. Cancer*, 1934, **20**, 48.

2 Burrows, H., *J. Path. and Bact.*, 1935, **41**, 43.

3 Lacassagne, A., *C. R. Soc. d. biol.*, 1935, **120**, 1156.

4 Zondek, B., *J. Exp. Med.*, 1936, **63**, 789.

5 Gardner, W. U., Allen, E., and Strong, L. C., *Anat. Rec.*, 1936, **64**, 17.

6 Perry, I. P., and Ginzton, L. L., *Am. J. Cancer*, 1937, **29**, 690.

the cut end fixed to the abdominal wall. One month later 500 I.U. of estrogen were injected subcutaneously and 7 days later bacteriological examination of the ligated and intact horns was made.

TABLE II.
The Incidence of Uteri Showing Bacteria in Mice Receiving Estrogen.

No. of mice	Treatment		No. with bacteria	No. without bacteria
	Duration	Total I.U.		
7	2 days	500	4	3
12	4 "	500	7	5
10	7 "	500	8	2
2	10 "	500 × 2	1	1
10	14 "	500	9	1
4	15 "	500 × 2	4	0
4	4 to 10 mo.	500 i.u. wkly.	4	0
9	2 to 7 days	2 to 10 i.u.	5	4

The mice were killed with illuminating gas and opened under aseptic conditions. The uterine horns were removed well above the cervix, ground in 0.5 cc. sterile saline and one loopful of the resulting suspension was streaked on heart-infusion blood agar and another inoculated into heart-infusion broth. Growth, if any, appeared in 12 to 24 hours. Negative cultures were examined for 5 days. Frequently colonies growing on the plates were subcultured and identified.

Of 45 control mice (5 immature, 18 mature virgin, 11 multipara, 7 castrated, and 4 mature virgins receiving oil) one mature virgin and one multipara showed bacteria in the uterus (Table I). Postmortem vaginal smears revealed early post-estrous stages in these 2 mice. The heart-bloods of 19 mice, 13 of which had received estrogen, were negative. The data on animals receiving estrogens are summarized in Table II. Bacteria were present in the uteri of some mice on the second day after a single injection of estrogen and in most of the uteri at the 7- and 14-day stages. The long-term mice had pyometra. Gram-stained sections revealed bacteria in many cases. The observations on the ligated uterine horns (Table

TABLE III.
Incidence of Bacteria in Intact and Ligated Uterine Horns.

No. of mice	Treatment duration	Total i.u.	Ligated horns				Intact horns			
			No. —		No. +		No. —		No. +	
			Broth	B.P.	Broth	B.P.	Broth	B.P.	Broth	B.P.*
13	7	500	7	12	6	1	2	2	11	11

*B.P. = blood plate.

III) indicated that infection occurred by direct extension through the cervix. Histological examination showed a correlation between the response of the tissues to estrogen and the presence of bacteria.

The types of organisms isolated varied in different animals. Most common were *Bact. alkaligenes* and an unidentified Gram-positive diplococcus, usually found together. *Bact. coli,* hemolytic and non-hemolytic streptococci and staphylococci were occasionally found. Frequently a single type of organism was obtained. Cultures of the vagina yielded similar organisms.

9584 P

Production of Phage in the Absence of Bacterial Cells.*

A. P. KRUEGER AND D. M. BALDWIN.

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In recent publications[1, 2] it was shown: (a) that during one phase of the reaction between phage and bacteria in the presence of 0.25M NaCl phage is formed without concomitant bacterial growth; (b) that when pH and temperature are properly adjusted the bacterial substrate can be maintained in the resting state while [phage] rises logarithmically with time at the rate of a ten-fold increase per hour.

The obvious inference of these experiments is that bacterial growth, long held to be the prime conditioning factor for phage-formation, is actually not essential at all. Instead it would appear quite possible that resting cells may produce some sort of phage-precursor which in the presence of phage is promptly converted into more phage. This hypothesis is supported to a considerable extent by our observations during the past 3 years on the increase in phage-titer that occurs when cell-free ultrafiltrates of bacterial suspensions are added to known quantities of phage. Young cultures of susceptible organisms are washed in saline solution to free them from phage-inhibitor.[3, 4] Dense suspensions of the washed cells are

* This work was supported by grants-in-aid of research from the American Medical Association and the Board of Research, University of California.

1 Scribner, J., and Krueger, A. P., *J. Gen. Physiol.*, 1937, **21**, 1.

2 Krueger, A. P., and Fong, J., *J. Gen. Physiol.*, 1937, **21**, 2.

3 Levine, P., and Frisch, A. W., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 993.

4 Burnet, F. M., *J. Path. and Bact.*, 1934, **38**, 285.

placed in infusion-broth of pH 7.4 at 36°C. for 2 hours. The fluid portion of the mixture is then separated from the cells by ultrafiltration through a 2% or 2.5% acetic-collodion membrane.[5] When equal quantities of the ultrafiltrate and known phage-preparations are mixed an increase in phage-titer occurs. For example when 1 ml. of ultrafiltrate is added to 1 ml. of phage containing 1×10^9 activity-units/ml.[6] the final titer remains 1×10^9 activity-units/ml. Such yields, 100% increases in the amount of phage originally added, are quantitatively significant inasmuch as the method of titration is capable of detecting differences in [phage] of $\pm$ 5%. Furthermore, when a solution of phage is subjected to serial dilution, using the ultrafiltrate as diluent, the actual titers are consistently higher than the concentrations calculated from the original amount of phage added and the difference between these values becomes progressively greater as the range of dilution is extended. That is, the phage-ultrafiltrate mixtures tend to maintain the original titer while plain phage diluted with broth or saline solution becomes increasingly weaker.

The fact that cell-suspension ultrafiltrates are active in producing this effect in only 80% of experiments has led us to a careful investigation of possible sources of error. Among these, 3 possibilities are worth mentioning in this preliminary paper.

A. Cultures occasionally become lysogenic and spontaneously produce phage without the addition of phage to the organisms. Our ultrafiltrates have been regularly tested for the presence of phage and to date have been entirely negative.

B. If bacterial cells pass through the ultrafilters their addition to solutions of phage along with the ultrafiltrate would naturally result in an increase in the phage-content of the mixtures. But the ultrafiltration-membranes are quite uniform, and rigorous testing of our ultrafiltrates for sterility has revealed no organisms in them.

C. It was considered that the apparent increase in titer might be due to some effect of the ultrafiltrate on the titrative setup. Phage is quantitatively determined by its activity. Consequently if the small quantities of ultrafiltrate carried over into the titration-tubes somehow stimulated the phage-producing mechanism, the time of lysis would be reduced and the mixtures would have a fictitiously increased original content of phage. Control-experiments have ruled out this possibility.

Most of our experiments have been independently repeated in this

[5] Krueger, A. P., and Ritter, R. C., *J. Gen. Physiol.*, 1929, **13**, 409.

[6] Krueger, A. P., *J. Gen. Physiol.*, 1930, **13**, 557.

laboratory by Mundell, Fong and Strietmann with essentially the same results. We do not know at this time what the substance is in ultrafiltrates of susceptible bacterial cultures which, when added to phage, apparently results in the production of more phage. However, a considerable mass of experimental data leads to the conclusion that normal bacterial cells produce some sort of a phage-precursor which is converted into phage by phage itself. A detailed description of such properties of the precursor as we have been able to determine will appear in another paper.

9585 P

"Glandular" Cells in the Pars Nervosa and Stalk of the Hypophysis.

I. Gersh. (Introduced by Carl G. Hartman.)

From the Department of Anatomy, Johns Hopkins University.

This note summarizes the results of an investigation of the possible glandular nature of the parenchymatous cells of the pars nervosa of the hypophysis. Functional histological analysis in rats of various ages under experimental conditions leads to the conclusion that these cells produce and secrete anti-diuretic substance for the control of the water metabolism of the animal.

This "glandular" cell has been seen in the pars nervosa of almost every class of mammals, and of pigeons and chickens. The cell is distinguished by the presence of granules or of lipoid droplets which were first seen in fresh mounts. The inclusions fill the cytoplasm, and extend out into the cell processes. These intracellular elements are of approximately the same size in any one cell, though they may vary somewhat in size in adjacent cells. Histochemical analysis of the droplets or granules shows that in the rat they are rich in neutral unsaturated fats; no other lipoids have been identified. In some other animals, the granules contain no visible lipoids. The inclusions are preserved best by Maximow's fluid in the mouse and white rat.

The characteristic features of the glandular cell appear very early in the rat's embryonic life, being easily recognizable in a 23 mm. embryo. The number and size of the cells and of their inclusion bodies increase throughout life to become most prominent in rats 2½ years old. The cells are distributed more or less evenly in the pars nervosa except at its junction with the hypophyseal stalk;

there they are closer together. They are more widely separated and smaller in the remaining portion of the stalk than in the glandular portion.

In any particular adult gland the glandular cells vary greatly in size. In spite of appreciable fluctuations in both their number and size in untreated rats of the same age, the cells fall into and form a "normal" range of variation. Fluctuations within this normal range have been correlated with dietary water intake in a manner which can be controlled experimentally. When rats are placed for 1-8 days on a dry diet containing 5% water, the glandular cells appear in greater number and in larger size than in control rats which have free access to water. When such dehydrated rats then have access to water for as short a time as one day, the number and size of differentiated cells revert to the normal range of untreated rats. A similar though less marked hypertrophy accompanies parturition. There is thus in the rat a correlation of cellular activity and morphology with a hypersecretion of the antidiuretic and perhaps also the oxytocic substances.

Not all the glandular cells in the pars nervosa are differentiated or characterized by their content of intracellular inclusions. Many are relatively undifferentiated. These may be transformed rapidly during hyperplasia under conditions such as those described above, to the more differentiated form. The two varieties of cells vary inversely in number.

The glandular cells are supplied with nerve fibers which sweep in bundles down the hypophyseal stalk. The nerve fibers and their endings on and around the special cells have been demonstrated by Dr. C. M. Brooks and the author. Evidence is accumulating in this laboratory that these are the cells affected by stalk (nerve) section, and that they degenerate in severe experimental diabetes insipidus induced by such section. There is evidence also that recovery from a "temporary" diabetes insipidus is accompanied by a transformation of the unaffected cells in the hypophyseal stalk into the more differentiated type.

9586

Comparative Effects of Congo Red and Liver Extract on Reticulocytes in Pigeons.

A. P. Richardson. (Introduced by P. J. Hanzlik.)

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The remissions produced in pernicious anemia by Congo red[1-4] are of interest, not only therapeutically, but also etiologically as regards the pathogenesis of the disease. Mermod and Dock[2] have used the antianemic action of this dye in support of the view that pernicious anemia is caused by a hemolytic toxin, and suggested that the remissions may be due to adsorption of the toxin on the colloidal surfaces of the dye. They reported that, in normal guinea pigs, intraperitoneal injection of Congo red caused an increase in reticulocytes just as did liver extract. This has been confirmed by Lassen, Jacobsen and Nielsen.[5] However, Wakerlin, Bruner and Kinsman[6] reported recently that Congo red did not increase reticulocytes in pigeons, but liver extract did. Since these authors injected the Congo red intramuscularly, and it is not absorbed readily from the tissues, due to its colloidal properties, and moreover is used clinically by intravenous injection, their report could not be considered final unless it agreed with more crucial tests. The object of this report is to present the results of such tests, that is, repeated intravenous injections of the dye, which guaranteed systemic action, compared with injections of liver extract under similar conditions. In addition, the range of reticulocytic variation was determined in normal pigeons which have been used considerably in bioassays of antipernicious anemia agents, so as to rule out possible errors in determining the reticulocytogenic actions of Congo red.

Healthy grain-fed pigeons were used throughout, each bird being confined in a separate cage. Daily reticulocyte counts were made according to Mermod's[7] method. Blood was obtained by punc-

1 Massa, M., and Zolezzi, G., *Klin. Wchnschr.*, 1935, **14**, 235.

2 Mermod, C., and Dock, W., *Science*, 1935, **82**, 155.

3 Lendvai, J., *Klin. Wchnschr.*, 1936, **15**, 1034.

4 Gualdi, A., *Riv. osp.*, 1936, **26**, 323.

5 Lassen, H., Jacobsen, E., and Nielsen, A., *Acta path. et microbiol. Scandinav.*, 1936, **13**, 543.

6 Wakerlin, G. E., Bruner, H. D., and Kinsman, J. M., *J. Pharm. Exp. Therap.*, 1936, **58**, 1.

7 Mermod, C., *J. Clin. Invest.*, 1936, **15**, 559.

ture of a small leg vein, using not over 0.05 cu. mm. Only those cells were counted as reticulocytes in which the reticulum completely surrounded the nucleus, as was done by Wakerlin, *et al.*[6] Congo red was used as a 1% solution in 5% dextrose solution, boiled for 5 minutes and then filtered through quantitative filter paper. This procedure avoids precipitation (aggregation) of the colloidal dye, which occurs in physiological and other salt solutions. Injections were made into a wing or leg vein. Liver Extract Concentrated—Lilly (N.N.R.) was injected intramuscularly. The effects of the 2 agents were compared by alternate injections in the same birds, and continued injections of Congo red alone in other birds for the continued periods of alternate injections. The injections were always preceded by 3-day periods of control reticulocyte counts.

Distribution of Reticulocytes. One hundred counts of reticulocytes were made in 30 normal pigeons and a distribution curve charted. The mean for the entire series was 7.8%, standard deviation, ±2.39, standard deviation of the mean, ±.239, and the probable error of the mean, ±0.1613. Thus, there was a considerable variation, but for the most part, the reticulocytes were grouped around 8.0%.

Congo Red and Liver Extract. The results on 3 groups of 9 pigeons are presented as curves of averages for the groups in Chart 1. The first group of 3 pigeons received 1.0 cc. of liver extract per kg., intramuscularly, daily for 5 days. There was a prompt rise in reticulocytes, followed by a fall to normal. At this point, 4 daily intravenous injections of Congo red (50 mg. per kg.) were given, total 200 mg. per kg. Daily reticulocyte counts were made until it was certain that no rise occurred. A second group of 3 birds received, after a control period, 4 daily intravenous injections of Congo red (50 mg. per kg.), total 200 mg. per kg., and, when it was apparent that no rise occurred, 5 daily injections of liver extract (1 cc. per kg.) were given intramuscularly. A third group of 3 birds received 4 daily injections of Congo red alone (50 mg. per kg.) intravenously, total 200 mg. per kg., and the counts continued for the same period as that of the other 2 groups.

The curves in Fig. 1 leave no doubt that Congo red was not reticulocytogenic in pigeons, when acting systemically, while liver extract produced the usual prompt rises, which reached a peak in about 6 days after the first injection. Thus, these crucial tests confirmed the claim of Wakerlin, *et al.*

It is worthy of note that the increase in reticulocytes in each bird

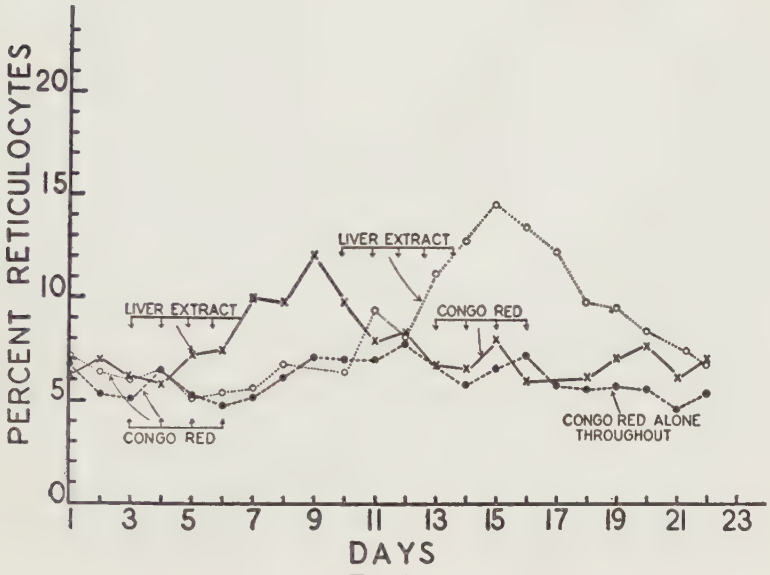

FIG. 1.

Comparative effects of Congo Red intravenously and liver extract concentrated intramuscularly on reticulocytes in normal pigeons.

Doses are given in the text; heavy bars indicate periods of medication in days, and arrows, individual doses of agents injected; each curve represents averages of results in 3 pigeons.

was not higher than that occurring occasionally in normal birds, and although the odds were very high that the response obtained was not due to chance variation, it would appear that the liver may produce only a response of such magnitude as might occur normally. In other words, liver extract may only be a "trigger", which sets off an otherwise normal variation. A number of agents are known to be reticulocytogenic for the pigeon, but ineffective therapeutically in pernicious anemia. Among these are extracts of beef steak[8] and normal urine,[10] and several amino acids.[9] In view of these facts, it is possible that the reticulocytogenic response to an impure material like liver extract may be due to some non-specific substance. Accordingly, the use of normal animals for bioassay of antipernicious anemia agents is unreliable and unsatisfactory.

On the other hand, the negative response of normal pigeons to Congo red does not mean that this dye may not be effective in pernicious anemia. For, if this disease is caused by a hemolytic toxin,

8 Vaughan, J. M., Muller, G. L., and Zetzel, L., *Brit. J. Exp. Med.*, 1936, **11**, 456.

9 Muller, G. L., *New Eng. J. Med.*, 1935, **213**, 1221.

10 Wakerlin, G. E., and Bruner, H. D., *Arch. Int. Med.*, 1936, **57**, 1032.

and Congo red can detoxify the toxin, then evidence of reticulocytogenic action, or of stimulation of bone marrow, could not be expected in a normal animal, where the toxin is not acting. It is possible that the toxin of pernicious anemia is not as readily detoxified by Congo red as are certain poisons and bacterial toxins.[11] The interesting fact remains that Congo red can act beneficially in a pathological state of the blood (pernicious anemia), and obviously non-specifically. Barker's recent negative report[12] is not final without more crucial and exhaustive tests.

Conclusions. 1. The variation of reticulocytes in normal pigeons has been determined. 2. Concentrated liver extract, injected intramuscularly, produced the usual rise in reticulocytes in normal pigeons, while Congo red, injected intravenously in the same and other pigeons for days, did not. This does not mean that Congo red can not act beneficially in pernicious anemia. The difficulties of bioassaying antipernicious anemia agents in normal animals are real and not always appreciated.

9587 P

Sensitization of Guinea Pigs to Cyclic Compounds and Effect on the Hematopoietic System.

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In recent years much experimental investigation of agranulocytosis has given rise to the view that "chemicals containing the benzene ring, may so depress the bone marrow that leucopenia results, and bacterial infection of the mouth may develop in consequence."[1] It has been the contention of Madison and Squier[2] that agranulocytosis is the result of drug *hypersensitivity* (to amidopyrine).

Landsteiner and Jacobs,[3] reported sensitization of guinea pigs to simple benzene ring compounds injected into, or spread on the skin of those animals for a number of consecutive days. Among those

11 Hanzlik and Butt, *J. Pharm. Exp. Therap.*, 1928, **33**, 260.

12 Barker, W. H., *Am. J. Med. Sci.*, 1937, **194**, 293.

1 Boyd, W., *The Pathology of Internal Diseases,* 2nd Ed., Lea & Febiger, Phila., 1935, p. 621.

2 Squier, T. L., and Madison, F. W., *Wis. Med. J.*, 1935, **34**, 175.

3 Landsteiner, K., and Jacobs, J., *J. Exp. Med.*, 1935, **61**, 643.

was 2:4 dinitro parachlor benzene. Accordingly, this procedure was repeated here, and blood studies made.

An alcoholic stock solution of 2:4 dinitro parachlor benzene was made, which when diluted with normal saline solution contained 1/500 mg. of the compound per 0.1 cc. Blood counts (r.b.c., w.b.c., and differentials) were made on all guinea pigs before, during, and after the injection period. Eight animals were used, 4 of which remained alive throughout the entire experimental period. Ffteen daily injections of 0.1 cc. each were made intracutaneously. The compound was found to be a slight irritant. Skin reactions, however, were read at all times by comparison with a previously uninjected albino guinea pig control. One month after the beginning of the injections each animal received 5 separate intracutaneous doses of 0.1 cc. each, and an albino control was similarly treated. The previously injected animals responded with marked zones of inflammatory edema at each injection site, a slight eosinophile rise, no significant change in the white blood cell count, no change in the red cell count, an increase in the lymphocyte percentage in three of the injected animals, and a decrease in the polymorphonuclear percentage in the same animals. The control animal's counts remained unchanged. Two weeks later the procedure was repeated using another albino control. The differential count in this instance was comparable to the level of these animals prior to the sensitizing procedure, with the exception of a slight eosinophilia. The red, and white cell counts remained unchanged. Seventeen days later each animal, and an albino control received 0.1 cc. of the solution intracutaneously. The procedure was repeated in 3 days, and blood counts were taken 6 days later. There was a slight decrease in the polymorphonuclear percentage and an increase in the lymphocyte percentage with no appreciable change in the red, or white cell counts. In each of the above instances the sensitized animals responded with marked zones of inflammation while the control showed no reaction, or one less than 2 mm. in diameter.

The route of injection was then altered. Nineteen days after the last injection each animal, and an albino control received 0.1 cc. of the diluted stock solution intracutaneously, 0.1 cc. subcutaneously, and 0.1 cc. intraperitoneally. No significant hematologic changes were observed.

The animals were observed for one month. Then each received intraperitoneally 1.5-2.0 cc. of an 18-hr. broth culture of an hemolytic *Staph. albus* in one instance; and a pneumococcus at another time. Both organisms were freshly isolated from human infections. After each inoculation each animal, and an albino control received

0.1 cc. of the solution of the chemical intracutaneously. The only response was a temporary leucocytosis. The animals were observed for another 2 weeks before concluding the experiment. No changes were observed.

9588

Ascorbic Acid Stimulation of Specific Antibody Production.*

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Jusatz[1] reported that oral administration of vitamin A, B, C, or D is without appreciable effect on the bactericidal titer of the blood serum of rabbits or on specific-antibody production in this animal species. However, intravenous injection of a massive dose of vitamin C (sodium salt of ascorbic acid) increased the bactericidal index about two-fold and specific-precipitin production about five-fold. We have attempted to repeat his experiments with ascorbic acid and to extend his antibody-stimulating studies to include other enzyme-activators, other animal species and other types of antigens. The present paper summarizes our initial confirmatory results with ascorbic acid introduced parenterally into rabbits during the process of active immunization against horse-serum proteins.

A total of twenty 2000 gm. control rabbits were each injected intravenously with 0.5 cc. of horse serum. An equal number of rabbits of the same size and weight were each injected intravenously with 0.5 cc. of horse serum plus 100 mg. of crystalline synthetic ascorbic acid (Merck). Each injected animal was bled from the ear vein at frequent intervals during the next 50 days and the resulting antiserums were titrated for antihorse precipitins. Composite data from the two groups are recorded in Fig. 1.

Ascorbic acid plus horse-serum proteins caused a prompter formation of specific precipitins than occurred in the control group injected with undenatured horse serum. The antibody-stimulating

* Work supported in part by the Rockefeller Fluid Research Fund of Stanford Medical School.

† Eli Lilly and Co. Research Associate in Bacteriology, Stanford University, California.

[1] Jusatz, H. J., *Z. f. Immunitätsforsch.*, 1936, **88**, 483.

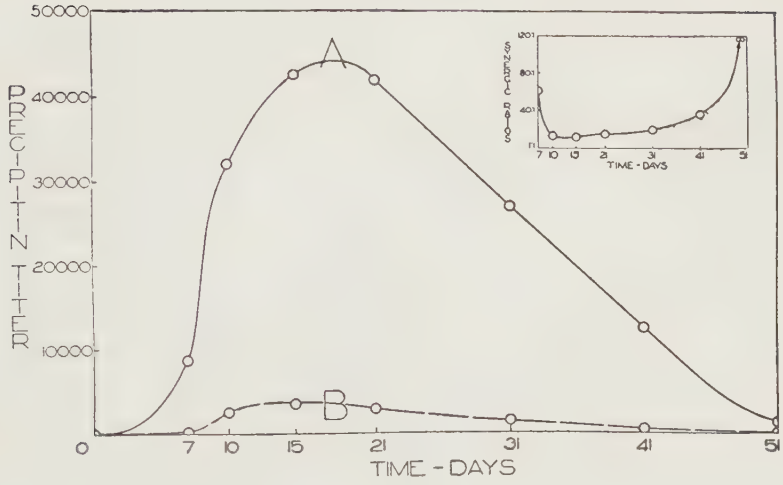

FIG. 1.

Vitamin C Stimulation of Specific Precipitin Production.

Precipitin-titers were determined as follows: 0.2 cc. of undiluted antiserum was overlaid with suitable dilutions of the antigen (horse serum). The tubes were then placed in the water bath at 37.5°C for 30 minutes and ring formation was observed. The tubes were then shaken and incubated for an additional period of 2 hours and then placed in the ice chest (4°C) overnight. Final readings were made the next morning. This is substantially the Lancefield technic.[2] The composite data were determined by averaging the ring-test titer and overnight readings and calculating a general average for the entire group of 20 animals. Recorded titers are expressed as precipitin-units per cc. of antiserum.

A. Composite data from twenty 2000 gm. rabbits, each injected intravenously with 0.5 cc. of horse serum mixed with 100 mg. of crystalline ascorbic acid (Merck).

B. Composite data from twenty 2000 gm. control rabbits each injected intravenously with 0.5 cc. of horse serum.

ratio for ascorbic acid when thus used is about 30:1 during the earlier stages of active immunization, falling to about a 12:1 ratio during the height of active immunity (14-21 days). Specific precipitins disappear from the control or non-vitaminized group by the fiftieth day, at which time the vitaminized group has a residual titer nearly equal to that of the control group at the height of active immunity.

In so far as the above tests were made with a purely arbitrary dose of ascorbic acid, an attempt was made to determine the optimal antibody-stimulating dose of this vitamin. Twenty-one 2000 gm. rabbits were divided into 7 groups of 3 each. Each group was injected intravenously with 0.5 cc. of horse serum plus varying amounts of ascorbic acid. The average 14- to 21-day precipitin-titers of the 7 groups are recorded in Fig. 2.

From this figure it is evident that massive intravenous doses of

2 Lancefield, R. C., *J. Exp. Med.*, 1933, **57**, 571.

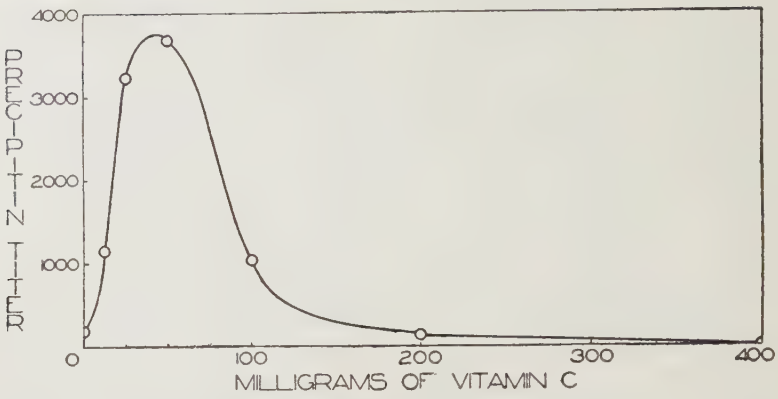

FIG. 2.

Determination of Optimal Antibody-stimulating Dose of Ascorbic Acid.

The curve shows changes in the average 14 to 21-day precipitin-titer in 7 groups of rabbits injected with varying doses of ascorbic acid mixed with 0.5 cc. of horse serum. Titers determined as in Fig. 1. The optimal antibody-stimulating dose is apparently midway between 25 and 50 mg. of ascorbic acid injected intravenously.

unneutralized ascorbic acid are toxic for rabbits, 400 mg., for example, completely suppressing specific-precipitin production. The optimal antibody-stimulating dose is apparently in the neighborhood of 37.5 mg. of ascorbic acid given intravenously. With this dose the average 14- to 21-day titer is about 20 times that of the control group.

The above result recalls the well-known differences between the antibody-stimulating and antibody-inhibiting doses of certain metallic salts reported by Walbum, *et al.*,[3] and of the alleged optimal prophylactic dose of vitamin C reported by Jungeblut and his associates in their studies of vitamin-C therapy in diphtheria[4] and poliomyelitis.[5]

To test the effect of variations in the site and method of injection of ascorbic acid, groups of about 4 rabbits were injected intraabdominally, subcutaneously, or endermally with arbitrary doses of horse serum or with the same dose plus arbitrary amounts of ascorbic acid. Several other small groups were given multiple in-

[3] Walbum, E. L., *Comp. rend. Soc. Biol.*, 1921, **85**, 761; *Z. f. Immunitätsforsch.*, 1926, **47**, 213; *Z. Tuberk.*, 1927, **48**, 193; *ibid.*, 1928, **51**, 209; *ibid.*, 1929, **53**, 292; *Z. f. Immunitätsforsch.*, 1929, **61**, 146; Walbum, E. L., and Mörch, J. R., *Ann. Inst. Pasteur*, 1923, **37**, 396; Madsen, T., and Mörch, J. R., *Acta Tuberc. Scand.*, 1926, **2**, 99; *Z. f. Hyg.*, 1928, **109**, 224.

[4] Jungeblut, C. W., and Zwemer, R. L., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1229.

[5] Jungeblut, C. W., *J. Exp. Med.*, 1937, **65**, 127.

jections with vitaminized or non-vitaminized horse serum. In all cases stimulation of precipitin-production was noted, the contrast being practically identical with those recorded in Fig. 1.

In other groups the method of injection was varied by injecting the horse serum and ascorbic acid separately, such as at different times in the same ear vein, or in different veins, or by giving horse serum intravenously and vitamin C intraäbdominally. Stimulation of specific-precipitin production was noted by all of these technics, confirming the conclusions of Burky[6] and of Swift and Schultz[7] in their studies of the immuno-"synergic"‡ effects of staphylococcal toxin. The antibody-stimulation, however, was less pronounced in these separate injections than those previously obtained by mixing the horse serum and ascorbic acid before injection.

The relative efficiency of ascorbic acid and its sodium salt was also compared in small groups of animals. Sodium ascorbate prepared by Sollmann's technic[9] was found to be but about half as effective as unneutralized ascorbic acid. Sodium ascorbate, however, is apparently unstable, a commercial preparation tested on a small group of rabbits being without demonstrable antibody-stimulating effect.

9589 P

Treatment of Human Pellagra with Nicotinic Acid.

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Pellagrins can be cured while on a maize diet by the oral administration of a filtrate of liver which contains the so-called "filtrate factor" but which is free from riboflavin and rat antidermatitis factor.[1]

[6] Burky, E. L., *J. Allergy*, 1934, **5**, 466.

[7] Swift, H. F., and Schultz, M. P., *J. Exp. Med.*, 1936, **63**, 703, 725.

‡ On presentation of this paper before the Pacific Coast Branch, Oct. 16, 1937, Dr. Swift's use of the word "synergic" was criticized by Dr. Tainter and other attending pharmacologists. In their opinion some variant of the word "potentiation" would be more nearly in accord with accepted usage.[8]

[8] Sollmann, T., *A Manual of Pharmacology*, W. B. Saunders Co., 5th Ed., 1936, p. 80.

[9] Sollmann, T., *ibid.*, p. 115.

[1] Fouts, P. J., Lepkovsky, S., Helmer, O. M., and Jukes, T. H., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 245.

The work of Jukes and Lepkovsky[2] indicates that the filtrate factor and the pellagra-preventing factor are probably not identical. Elvehjem, Madden, Worley, and Strong[3] have recently isolated nicotinic acid amide from a liver concentrate which cures canine blacktongue. Both the material from the liver concentrate and a commercial preparation of nicotinic acid cured blacktongue in dogs. Lepkovsky and Jukes,[4] however, have been unable to substitute nicotinic acid for either the filtrate factor or the rat antidermatitis factor in their studies on chicks and rats. Helmer and Fouts[5] likewise in studies on rats have not been able to replace the filtrate factor with nicotinic acid. These studies, therefore, indicate that the liver filtrate used in previous studies contains at least 2 active components.

It is the purpose of this paper to record the results of feeding nicotinic acid to 4 pellagrins. On admission to the hospital the patients were placed on a maize diet similar to the one described by Spies.[6] During the 3 or more days of the control period their condition either remained stationary or became worse. After this period one patient received one gram of nicotinic acid daily while the others received 500 mg.*

All patients showed distinct improvement in general condition and mental attitude within 48 hours of onset of therapy. The stomatitis of one patient showed beginning regression within 24 hours while in the others healing of the stomatitis was definite within 48 hours. Stomatitis was completely healed within 4 days in 3 patients and 5 days in the fourth. Excessive salivation which was present in only one patient decreased within 72 hours and had completely disappeared in 13 days. Severe diarrhea was present in 2 patients. The stools were less frequent and of more normal consistency within 24 hours in one and within 48 hours in the other. The stools were normal within 72 hours in one and by the fifth day in the other. The dermatitis increased during the first 24 hours in one patient but healing was initiated during the succeeding 24 hours. There was distinct abatement in the dermatitis within 48 hours in 2 of the other patients and within 4 days in the fourth. The dermatitis of the 4

2 Jukes, T. H., and Lepkovsky, S., *J. Biol. Chem.*, 1936, **114**, 117.

3 Elvehjem, C. A., Madden, R. J., Worley, D. W., and Strong, F. M., *J. Am. Chem. Soc.*, 1937, **59**, 1767.

4 Lepkovsky, S., and Jukes, T. H., unpublished data.

5 Helmer, O. M., and Fouts, P. J., unpublished data.

6 Spies, T. D., *J. Clin. Invest.*, 1934, **13**, 807.

* The nicotinic acid was supplied in part by Mr. George B. Walden of Eli Lilly and Company.

patients had disappeared by the sixth, fourteenth, twenty-second and twenty-fifth days on therapy. Two of the patients developed neuritic pains in the lower extremities while the symptoms of pellagra were improving. All patients noted sensation of heat and tingling of skin within 10 minutes after ingesting nicotinic acid. These sensations lasted for 10 to 20 minutes. During this time there was distinct dilatation of peripheral blood vessels but only slight temporary fall in blood pressure.

Summary. Improvement in 4 patients with pellagra following administration of nicotinic acid was as satisfactory as that following administration of liver filtrate except for an increase in time required for complete disappearance of dermatitis.

9590 P

Hypoglycemic Action of Alloxan.

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The study of the physiological regulation of blood-sugar concentration embraces a complexity of independently variable factors whose individual influences are still incompletely understood. Many experimental substances and conditions will cause hyperglycemia, but none besides insulin are known which will cause hypoglycemia.

Alloxan will produce hypoglycemia in normal rabbits in a very characteristic fashion. If upwards of 70 mg. of alloxan monohydrate per kg. of body weight is injected intravenously the blood sugar level of the animal will fall below the normal value (less than 70 mg. %) in about 3-4 hours, and will continue to fall steadily during the next 2-4 hours until the convulsive level (less than 35 mg. %) is reached. It will keep falling further during the convulsive stage until the animal expires, and very low (less than 15 mg. %) values may be observed terminally. All normal rabbits respond in the same way, although some delay in the time of onset of convulsions is observed if the animals have not fasted for 12-24 hours before the experiment. The larger dosages of alloxan (150-200 mg./kg.) will not hasten the appearance of convulsions, but will very definitely cause the recurrence of convulsions after remissions induced by glucose.

Both the convulsions and the hypoglycemia are promptly relieved

by glucose intravenously administered. Animals in violent convulsions will return to a conscious and quite normal state within one minute after glucose has been given. After 5-10 minutes they will eat and drink in a normal manner. If the dosage of alloxan has been large hypoglycemia and convulsions may recur several times at intervals of 2-3 hours provided that each attack is treated with glucose. Large dosages of glucose or sufficient food intake tend to forestall subsequent seizures. The effect of alloxan persists for at least 24 hours in fasting animals.

The graphs in Fig. 1 illustrate the effect of alloxan on the blood-sugar concentration. Alloxan was dissolved in water and given to young adult rabbits intravenously. All the animals except No. 6

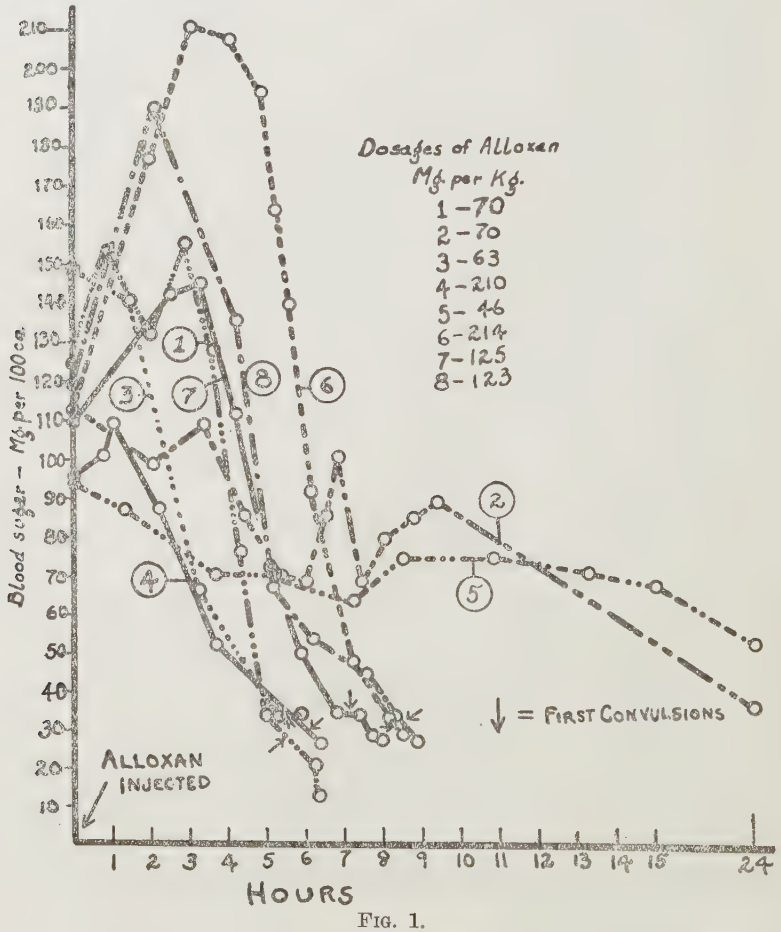

FIG. 1.
The Effect of Alloxan on the Blood-Sugar Level of Normal Rabbits.

fasted for 12 hours before the experiment. The first point on each curve gives the value for the blood-sugar concentration just before the injection. Blood sugar estimations were made by the method of Miller and Van Slyke[1] on samples drawn from the ear veins. Each curve represents an experiment with a fresh animal. Whether the transient hyperglycemia which precedes the fall in blood-sugar concentration in some of the animals is a specific effect of alloxan cannot be stated.

That the effects of alloxan are largely a consequence of the lowering of blood-sugar concentration is indicated by the specific antidotal action of glucose. Furthermore, after the acute effects of alloxan have subsided, the animals treated with it return rapidly to a normal state. In fatal experiments a marked rigor appears at once, but all the organs, and particularly the liver, look normal grossly.

In order to try to learn whether the described effects are due to alloxan itself or to one of its simpler decomposition products several likely derivatives of alloxan were also tested. Alloxanic acid, dialuric acid, isodialuric acid, barbituric acid, isobarbituric acid, alloxantin, murexide, mesoxalic acid, parabanic acid, oxaluric acid, formyl-oxaluric acid and formylurea exhibited no effects similar to those of alloxan when given to rabbits in substantial doses. As Cerecedo[2] noted, formylurea and formyl-oxaluric acid were extremely toxic.

At present there is no explanation for the hypoglycemic action of alloxan. There is no evidence to indicate that alloxan is chemically or physiologically related to insulin, or that the mechanism by which it produces hypoglycemia is a physiological one. Alloxan is an oxidizing agent credited with special affinity for the hydrogen of sulphydryl groups.[3-6] It is rapidly changed to alloxanic acid by alkalis. Insulin is sensitive to reducing agents and to alkalis. Whether the capacity for being readily reduced chemically, which is possessed by both alloxan and insulin, is related to the effect on the blood-sugar concentration remains to be settled. Labes and Friedberger[5] regard alloxan as a capillary poison.

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1 Miller, B. F., and van Slyke, D. D., *J. Biol. Chem.*, 1936, **114**, 583.

2 Cerecedo, L. R., *J. Biol. Chem.*, 1931, **93**, 269.

3 Strecker, A., *Ann. d. Chem.*, 1862, **123**, 363.

4 Wieland, H., and Bergel, F., *Ann. d. Chem.*, 1924, **439**, 196.

5 Labes, R., and Friedberger, H., *Arch. exp. Path. u. Pharmakol.*, 1930, **156**, 226.

6 Lieben, F., and Edel, E., *Biochem. Z.*, 1932, **244**, 403; 1933, **259**, 8.

9591 P

Effect of Hypophysectomy on Glycogen Distribution in Tumor-Bearing Rats.*

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In an experiment in which the effect of pituitary removal on the rate of growth of Walker tumor No. 256 was compared with the effect of caloric restriction in intact tumor-bearing male rats, samples of liver, muscle, and tumor tissue were secured for glycogen determinations.

All animals had been fed by stomach tube with a formula composed of

Powdered whole milk	100 gm.
Glucose	100 "
Harris Yeast Extract	15 "
Water to	300 cc.

The hypophysectomized animals received twice daily throughout the experiment 1 cc. of this mixture per 40 gm. of body weight, calculated at the beginning of the experiment when tumor weight was negligible. The amount of food received by the controls was varied to maintain the total weight (somatic weight plus tumor weight) roughly equivalent to that of the hypophysectomized rats. This resulted in a restriction to approximately ¾ of the normal food intake in the intact tumor-bearing animals, while the hypophysectomized animals were given more food than they would have voluntarily consumed. At the time the glycogen samples were secured the hypophysectomized group had been deprived of pituitary tissue for 21 days. Liver, muscle, and tumor samples were taken in the order mentioned under amytal anesthesia 4 hours after the last feeding, and were frozen, weighed, and digested in the usual manner. Analyses were made according to the method of Good, Kramer and Somogyi[1] except that, in addition, the precipitate was washed once with 60% alcohol after being drained. The wash alcohol was also carefully drained off. The sugar in the final hydrolysate was determined by the Shaffer-Hartmann-Somogyi method. The percentage of glycogen present in the sample was calculated

* Aided by a grant from the International Cancer Research Foundation.

1 Good, C. A., Kramer, H., and Somogyi, M., *J. Biol. Chem.*, 1933, **100**, 485.

TABLE I.

Glycogen Values for Livers, Muscles, and Walker No. 256 Tumors in Post-Absorptive Hypophysectomized and Intact Male Rats.

	No. Animals	Aver. Tumor Size in gm.	Livers %	Muscles %	Tumors %
Intact	11	21.8±1.9	2.42±0.23	0.295±0.023	0.026±0.0017
Hypophysectomized	7	6.04±0.49	2.32±0.23	0.367±0.013	0.046±0.0025
Ratio of Difference to Probable Error of Difference		8.0	0.31	2.8	6.7

TABLE II.

Glycogen Values for Livers, Muscles and Walker No. 256 Tumors in Hypophysectomized and Intact Rats on a High Caloric Intake.

	No. of Animals	Average Tumor wt., gm.	Livers %	Muscles %	Tumors %	Tumors mg./cm.^{2}
Intact		8.68±0.56				
Post-absorptive	7		2.59±0.14	0.318±0.032	0.058±0.008	0.095±0.017
Fasted 28-30 hrs.	4		0.0279±0.0034	0.157±0.0055	0.055±0.026	0.088±0.011
Hypophysectomized						
Post-absorptive	5	4.06±0.91	3.03±0.34	0.222±0.012	0.186±0.015	0.26±0.014
Ratio of Difference to Probable Error of Difference (groups 1 and 3)		4.3	1.19	2.8	7.5	7.5

from this figure. For the tumors, glycogen was expressed as percentage of the total weight, or as mg. per sq. cm. of surface.

The data in Table I reveal that the small slow-growing tumors of the hypophysectomized animals contain significantly larger amounts of glycogen than the tumors of the intact group, while the liver and muscle values are not significantly different.

Another experiment was done in which both groups of animals received a high caloric diet by stomach tube. In this instance the tumors of the hypophysectomized animals contained about 4 times as much glycogen as in the first experiment while the tumors of the unoperated controls showed only twice as much as formerly (Table II). In this instance there was not as marked a difference in tumor size as formerly, and the sampling of the tumors of both groups was more uniform. Since tumor necrosis is preponderantly central it is perhaps preferable under these circumstances to express the glycogen values as a function of the surface. In the first experiment this was not done, since only peripheral portions of the large control tumors were used for analysis whereas the small tumors of the hypophysectomized animals were used *in toto*.

The question of the nature of the glycogen in the tumors naturally arises. Is it purely a storage phenomenon? The values for the fasted intact animals indicate that whereas large proportions of the liver and muscle glycogen may be lost through fasting, the tumor glycogen concentration remains at the same level as formerly, a level almost twice that found in the livers of this group. It appears, then, that the tumor glycogen fraction is not a labile one. We have neither a satisfactory explanation of why glycogen values are higher in tumors of hypophysectomized animals nor of what the presence of this glycogen means in terms of tumor metabolism.

9592 P

A Comparison of the Ketolytic Effect of Succinic Acid with Glucose.

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Korányi and Szent-Györgyi[1] have reported recently that succinic acid given as the calcium salt in daily amounts of 5 to 10 gm. or less is able to decrease the ketosis in diabetics. In the present investigations we have compared the ketolytic action of succinic acid with that of glucose when administered twice daily to fasting male rats weighing approximately 200 gm., in which the endogenous ketonuria was induced by the previous administration of a high butter-fat diet low in protein, as reported earlier.[2] The studies on ketonuria were made on the second to fifth fast days inclusive during which sodium chloride solution (fasting controls), sodium succinate in doses from 9.95 to 149.2 mg. per 100 gm. rat daily, or glucose in equivalent amounts (7.55 to 113.2 mg. per 100 gm. rat) was fed. Urine collections were made daily. The ketonuria of the fasting controls usually rises to a maximum on the third fast day, after which it diminishes rapidly. In Table I a summary of the data is

TABLE I.
Ketolytic Action of Glucose and Succinic Acid.

Fasting Controls		Glucose			Sodium Succinate		
(1)	(3)	(1)	(2)	(3)	(1)	(2)	(3)
16	49.2	16	7.55	45.8	14	9.95	52.1
40	33.7	36	37.8	19.4	29	49.7	38.1
16	29.1	14	75.5	10.0	11	99.5	28.2
11	21.5	14	113.2	5.2	11	149.2	21.5

(1) Number of experiments.
(2) Dose in mg. per 100 gm. rat.
(3) Acetone bodies as acetone in mg. per 100 gm. rat.

given. No ketolytic effect obtained when as much as 149.2 mg. of succinic acid per 100 gm. rat was given although glucose at a comparable level gave a marked decrease in the acetonuria. This amount of succinic acid on a surface area basis is 50% greater than

1 Korányi, A., and Szent-Györgyi, A., *Orvosi Hetilap*, 1937, **81**, 615. Cited from *Chemical Abstracts*, 1937, **31**, 6335.

2 Deuel, H. J., Jr., Hallman, L., and Murray, S. *J. Biol. Chem.*, 1937, **119**, 257.

the largest dose employed by Korányi and Szent-Györgyi. A slight decrease in ketonuria appears when only 7.55 mg. of glucose is given while a dose of 37.8 mg. caused an average decrease of acetone-bodies in the urine from 33.7 mg. per 100 gm. rat in the control tests to 19.4 mg. There was no evidence of diarrhea in any experiments nor of other toxic effects ascribable to the succinic acid. It is concluded that succinic acid is ineffective in preventing the ketonuria in fasting rats previously fed a high fat diet when this acid is administered in amounts far in excess of the quantity of glucose required to bring about a marked lowering in the excretion of the ketone bodies.

9593

Effect of Feeding and Fasting on Sugar Utilization of Eviscerated Rabbits.

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In an earlier paper by one of us[1] it was shown that the eviscerated rabbit utilizes glucose at a rate that is quite definite and fairly constant for a given animal. The rate varies, however, between different rabbits. The suggestion was made in that paper that this variation was due to the differences in the degree of fasting to which the different animals had been subjected prior to operation and that the effect of fasting is to reduce the utilization rate of the eviscerated animal. The work here reported was planned to investigate this relationship. The same technique was used as in the previous work. Essentially this consisted of measuring the rate at which glucose had to be administered to the eviscerated rabbit to maintain the blood sugar at a constant normal level.

Frequent blood sugar determinations served as a guide to the injection rate; if the blood sugar level rose the rate would be diminished, and *vice versa*. The determination was started 3 hours after completion of the operation and continued for 4 hours thereafter. Only those animals that sat up in normal posture and had normal righting reflexes after operation were used. The animals were also tested for kidney function by injecting phenol red after the opera-

[1] Drury, D. R., *Am. J. Physiol.*, 1935, **111**, 289.

TABLE I.
Rate of Utilization of Glucose in mg. per kilo per hour.

Fasted	Fed
70	137
81	175
85	191
97	205
110	208
110	209
112	221
133	228
138	236
166	246

tion. Any animals not secreting this dye were eliminated from the series. Ten rabbits were used in each of the two groups—one group fed to the time of operation, and the other fasted 4 to 6 days prior to operation. The glucose utilization rates are given in Table I.

The rates are given for the period between the 3rd and the 7th hours after operation. In this way we could be sure that we were not getting any anesthetic effect. However, the rate varies very little, as is shown in the previous paper.

The last 3 animals of the fasted group had rates of less than 110 mg. during the first 5 hours, after which they increased. They had excreted phenol red by that time so we felt they should be included in the series although kidney action may have stopped at that time. Despite the inclusion of these, there is undoubtedly a difference in the rate of the two types of preparation. The average for the fed group is 206 and that for the fasted 110. There is overlapping of individual rates in the two groups in just one case.

Is this difference due to insulin? It is reasonable to suppose that in a fed animal the islet tissue is more active than in a fasted one and although the operation involves complete removal of the pancreas there would probably be more insulin circulating in the fed than in the fasted, immediately after operation. It seemed necessary, therefore, to determine the duration of action of insulin in the eviscerated preparation and we investigated this in a separate series of animals. After evisceration we determined the glucose utilization rate. We then injected insulin intravenously and redetermined the utilization rate during successive periods thereafter.

The following is a typical protocol:

Rabbit fasted 6 days prior to operation.

9:45 A.M.	Operation completed.
11:15-12:16	Glucose utilization rate 101 mg. per kilo per hr.
12:40	0.025 U insulin intravenously.
12:55-1:31	Glucose utilization rate 172 mg. per kilo per hr.
1:31-2:34	" " " 97 " " " " "
2:34-3:58	" " " 69 " " " " "

This procedure was carried out on 9 other rabbits. The results are given in Table II.

TABLE II.

Days Fasted	Utilization rate before insulin	Dose of insulin units	Utilization rate immediately after insulin	Utilization rate later	Time after insulin of 2nd determination hrs.
5	110	.10	170	133	2
4	94	.10	185	138	3½
5	82	.10	188	108	6½
6	67	.050	150	111	2
4	107	.050	229	144	3½
6	53	.050	143	80	2
5	—	1.00	249	150	4½
Not fasted	211	.10	357	242	4½
" "	176	.10	285	174	2½

It is apparent from these results that the effect of these doses of insulin is largely over in 3 or 4 hours. The higher glucose utilization rates which we obtained in fed animals for 7 hours after operation could not have been due to insulin which was in the body at the time of operation. The main effect would have worn off long before that. It might be claimed that a very large amount of insulin was present in the fed animals at the time of operation; but this would have given a very high utilization rate in the first 2 hours after evisceration. To test this we injected 5 units into such a rabbit and obtained a utilization rate of over 600 mg. per kilo per hour during the first 2 hours after injection. As stated previously in this paper and as shown in the previous article[1] the utilization rate in fed animals immediately after evisceration is not that high but is the same as that shown 5 to 7 hours after that. We cannot then explain this high rate of glucose utilization by fed rabbits after evisceration as an insulin effect.

It is more probable that the difference between the glucose utilization of the fasted and fed animals is due to action of some other gland such as thyroid, pituitary or adrenal. There is much experimental work which suggests that the secretions of these glands have an action on glucose metabolism and that their action is more lasting than insulin. Thus Greeley[2] finds that the increased utilization of glucose in hypophysectomized rabbits does not occur until 20 hours on an average after removal of the gland. This would suggest a glucose-utilization inhibiting hormone of the pituitary which has quite a prolonged action.

2 Greeley, P. O., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1070.

We may conclude then that glucose metabolism is constantly modified by at least 2 hormonal mechanisms. The agent of the first of these is insulin, powerful but acting relatively a short time (2 to 3 hours for a given medium dose). The other is a mechanism having a longer duration. The insulin mechanism may be considered as taking care of immediate needs, responding very quickly to eating of food or rise in blood sugar but having a relatively transitory effect for one given dose. The other mechanism is more slowly produced but acts during a prolonged period.

Summary. The rate of utilization of glucose by the eviscerated preparation is affected by feeding and fasting prior to operation. The tissues of the fed animals utilize glucose at a rate double that of the fasted.

This increase cannot be due to insulin.

We wish to acknowledge our appreciation to the Eli Lilly Company for the gift of insulin used in these experiments.

9594

Recovery from the Anemia Caused by a Diet Deficient in Vitamin K.

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The purpose of this investigation was to ascertain whether the restoration to normal of the clotting time of chicks on a diet deficient in Vitamin K was accompanied by a cure of the existing anemia. This anemia has been observed by Dam,[1] and Holst and Halbrook,[2] but thus far no extensive investigations on the regeneration of hemoglobin have been made. The changes toward normal produced by the administration of minute quantities of a Vitamin K concentrate were so rapid that it seemed desirable to report our results.

The chicks used in our experiments were one-day-old white Leghorns hatched from eggs laid by hens which had been fed a diet relatively free from Vitamin K. They were raised in battery brooders and had free access to food and water. The basal diet used in these experiments was that of Almquist[3] with only minor

1 Dam, H., *Biochem. Z.*, 1929, **215**, 475.

2 Holst, W. F., and Halbrook, E. R., *Science*, 1933, **77**, 354.

modifications. Our active extract was prepared from alfalfa meal by an adaptation of the methods of Dam and Schønheyder[4] and of Almquist.[3]

Blood was drawn from the brachial vein, samples being taken for the determination of clotting time, hemoglobin, and the erythrocyte count. The clotting time (Schønheyder[5]) was determined by allowing the blood to flow directly into a small porcelain dish. Zero time was taken as the time when the first drop fell into the dish. When the blood no longer flowed upon tilting the dish it was considered to be clotted. The blood was observed for 300 minutes, if it failed to clot in a shorter time. The hemoglobin was determined by the method of Palmer[6] in which the hemoglobin is converted to carbon monoxide hemoglobin and compared with a standard solution of carbon monoxide hemoglobin in a colorimeter. The erythrocyte count was made in the conventional manner.

The first group of 12 normal chicks was placed on a normal diet consisting of a commercial preparation (Startena—Ralston Purina Mills) for 35 days. From this group we obtained the normal values (Table I). The second group of 12 chicks was kept on the basal diet No. 12 supplemented with 0.5% dehydrated alfalfa meal for 35 days. The third group of 21 chicks was placed on the basal diet No. 12, five of them being kept on the diet for 27 days (Sub-Group A, controls) and 16 for 35 days (Sub-Group B, controls). All of this group of chicks were given 0.35 cc. of oleum sesami daily for the last 3 days. The fourth group of 21 birds was kept on the basal diet No. 12, five of them for 27 days (Sub-Group A, treated) and 16 for 35 days (Sub-Group B, treated). On the last 3 days the birds received 0.2 cc. oleum sesami containing 1.0 mg. of an alfalfa extract 4M8a daily. This solution was administered from a 1.0 cc. tuberculin syringe connected to a tube 9 cm. in length and 1.0 mm. in diameter. The tube was inserted directly into the crop, and the solution of the extract was washed through the needle with an additional 0.15 cc. of sesame oil. The results indicate that oleum sesami is a suitable vehicle in which to administer the active extracts since the controls not receiving Vitamin K showed the profound anemia and prolonged clotting time.

It is clear from the results obtained with the second group of birds that 0.5% alfalfa meal adequately protects the birds from

[3] Almquist, H. J., *J. Biol. Chem.*, 1936, **114**, 241.

[4] Dam, H., and Schønheyder, F., *Biochem. J.*, 1936, **30**, 897.

[5] Schønheyder, F., *Biochem. J.*, 1936, **30**, 890.

[6] Palmer, W. W., *J. Biol. Chem.*, 1918, **33**, 119.

TABLE I.

Diet	No. of Chicks	Clotting time in minutes			Erythrocytes per cu.mm. in millions			Hemoglobin gm./100 cc.		
		Min.	Max.	Aver.	Min.	Max.	Aver.	Min.	Max.	Aver.
Commercial feed (Startena), normal chicks	12	1.0	6.0	3.60	2.57	3.60	2.98	8.16	11.25	9.49
Diet 12 plus 0.5% dehydrated alfalfa leaf meal	12	1.5	6.0	3.5	2.30	3.07	2.67	7.47	9.72	8.92
Diet 12 (Controls)										
Sub-Group A	5	13	>300	78	1.80	2.01	1.94	6.91	8.77	7.79
Sub-Group B	16	14*	>300	266	0.90	2.10*	1.50	2.52	9.18*	5.68
Diet 12 plus 3 mg. of alfalfa extract 4M8a										
Sub-Group A	5	1	2.5	1.60	2.40	2.87	2.65	7.50	9.24	8.26
Sub-Group B	16	0.75	5.0	2.48	2.14	3.30	2.71	5.50	9.49	8.25

*These values were obtained from the same bird.

Vitamin K deficiency. With one exception all of the birds in the third group developed the deficiency to a marked degree. The clotting time was prolonged and the anemia severe, in one case the red cell count being as low as 900,000 per mm.^{3} and the hemoglobin 2.5 g. per 100 cc. of blood.

The 5 birds kept on the diet for 27 days showed an average clotting time of 78 minutes. Of the birds kept on the diet for 35 days only 2 showed clotting time of less than 300 minutes; however, one of these showed almost normal values for clotting time and hemoglobin. Even under extreme conditions an occasional bird fails to develop severe deficiency.

The birds of the fourth group receiving the extract containing the antihemorrhagic factor for only 3 days showed values for erythrocytes and hemoglobin which were approximately normal, and an average clotting time of 2.3 minutes. The return to normal accomplished by the administration of minute quantities of Vitamin K for 3 days shows that the hematopoietic tissues must have been functioning at an amazing rate.

In a recent examination of a standard grade of fish meal Almquist and Stokstad[7] obtained no evidence of the presence of toxic substances which prolong clotting time or produce anemia. According to these investigators[8] the anemia of chicks on a diet deficient in Vitamin K "must be regarded entirely as a consequence of hemorrhage." While there is no doubt that hemorrhage is a very important factor in the anemia it is possible that other factors play a rôle. Since it is conceivable that an examination of the hematopoietic tissues may throw additional light on the anemia, Dr. G. O. Broun is conducting a study of the bone marrow.

Conclusions. The administration of Vitamin K to chicks showing a prolonged clotting time and a profound anemia restores both of these to normal values. Minute amounts of Vitamin K permit complete recovery from the anemia within a period of 3 days.

We wish to acknowledge financial assistance from the Theelin Fund administered by the Committee on Grants for Research of St. Louis University.

7 Almquist, H. J., and Stokstad, E. L. R., *Poultry Science*, 1937, **16**, 261.

8 Almquist, H. J., and Stokstad, E. L. R., *J. Nutrition*, 1937, **14**, 235.

9595

Action of Zinc on Effect of Adrenalin Given Subcutaneously.

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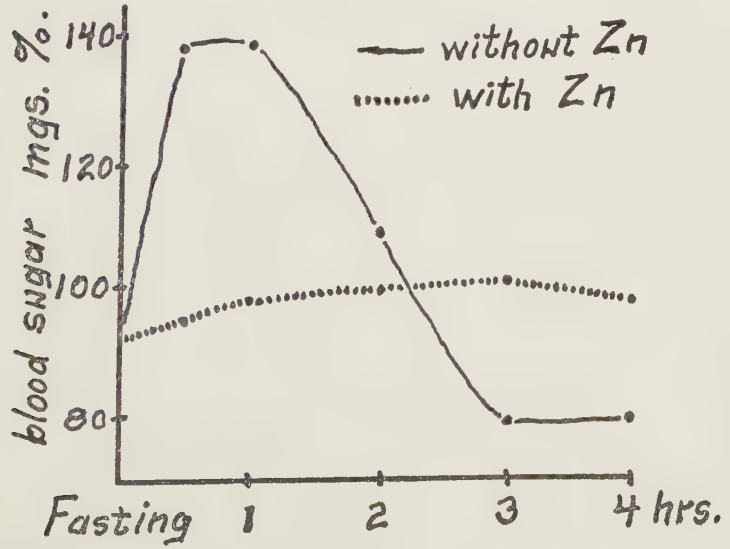
The interest in the effect of zinc on the action of insulin and various other hormones stimulated a study of its effect on the activity of adrenalin. The subjects were patients at rest in bed and in the post-absorptive state. Capillary blood sugar and blood pressure were determined before and for four hours after subcutaneous injection of 0.5 mg. of adrenalin hydrochloride (1-1000 solution). Curves for comparison were made with and without the added zinc, using symmetrical areas for injection and intervals of 2 to 3 days between examinations.

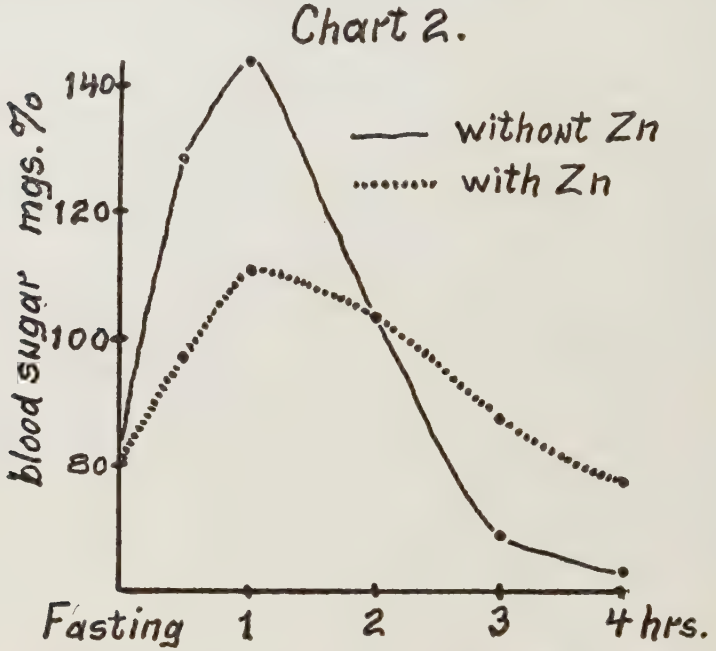
In the first series equal amounts of zinc and adrenalin were used. The zinc was added, immediately before injection, as 0.1 molar solution of zinc sulphate. Correction was made for the small increase in volume. The average blood sugar curves for 9 subjects are shown in Chart 1. The marked decrease in response is evident.

In the second series the effect of one-tenth as much zinc as adrenalin was studied. (0.05 mg. of zinc was given with 0.5 mg.

Chart 1.



of adrenalin. This is about the same order of magnitude as the concentration of zinc in commercial protamine zinc insulin.) The zinc was added as 0.01 molar zinc sulphate solution. The average blood sugar curves for 6 subjects are shown in Chart 2. The decrease in response to adrenalin is again evident.



Average blood pressure curves were similar to the blood sugar curves but, in individual cases, more irregular and not always consistent.

With small amounts of zinc the total activity of adrenalin appears to be definitely diminished. Since adrenalin is oxidized rapidly in the body, it seems probable that, under these conditions, it has been absorbed more slowly and therefore a greater proportion oxidized in a given interval of time.

9596

Progestin in the Pregnant Mare

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The high unitage of gonadotropin in the blood[1] and especially the placenta[2] of the mare during the second, third and fourth months of pregnancy when the fetus is growing from about 2 to 20 cm. (crown-rump length) would seem to be responsible for the secondary, multiple luteal bodies of the maternal ovaries,[3] and the remarkable overgrowth of the fetal gonads.[4] The primary corpus of pregnancy regresses at about the end of the first month, and from then until about the end of the fourth month multiple corpora are found in both ovaries. Cole and others[3] found rupture points in some of the secondary corpora, leading them to the belief that these bodies followed a true ovulation as normally, but histologic studies have led us to believe that the secondary corpora may also be formed by hyperplasia of the theca interna with accompanying degeneration of the ovum and granulosa (Fig. 1). Such theca-luteal bodies may be produced in rodents by injections of pregnant mare's gonadotropin, and appear similar to the aberrant corpora of rhesus monkeys.[5]

The secondary corpora usually regress by mid-pregnancy, and the maternal ovaries become atrophic and fibrotic. It was of interest, therefore, not only to determine the progestin-content of the corpora lutea, but also to investigate other possible sites of production or storage of that hormone, especially the placenta in the latter half of pregnancy, since some workers[6, 7, 8] have found progestin in the human placenta. Unfortunately the work on the detection of pro-

1 Cole, H. H., and Hart, G. H., *Am. J. Physiol.*, 1930, **93**, 57.

2 Catchpole, H. R., and Lyons, W. R., *Am. J. Anat.*, 1934, **55**, 167.

3 Cole, H. H., Howell, C. E., and Hart, G. H., *Anat. Rec.*, 1931, **49**, 199.

4 Cole, H. H., Hart, G. H., Lyons, W. R., and Catchpole, H. R., *Anat. Rec.*, 1933, **56**, 275.

5 Corner, G. W., Bartelmez, G. W., and Hartman, C. G., *Am. J. Anat.*, 1936, **59**, 433.

6 Ehrhardt, K., *Münch. Med. Wchnschr.*, 1934, **81**, 869.

7 Adler, A. A., Fremery, P., and Tausk, M., *Nature*, 1934, **133**, 293.

8 McGinty, D. A., McCullough, N. B., and Wolter, J. G., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 176.

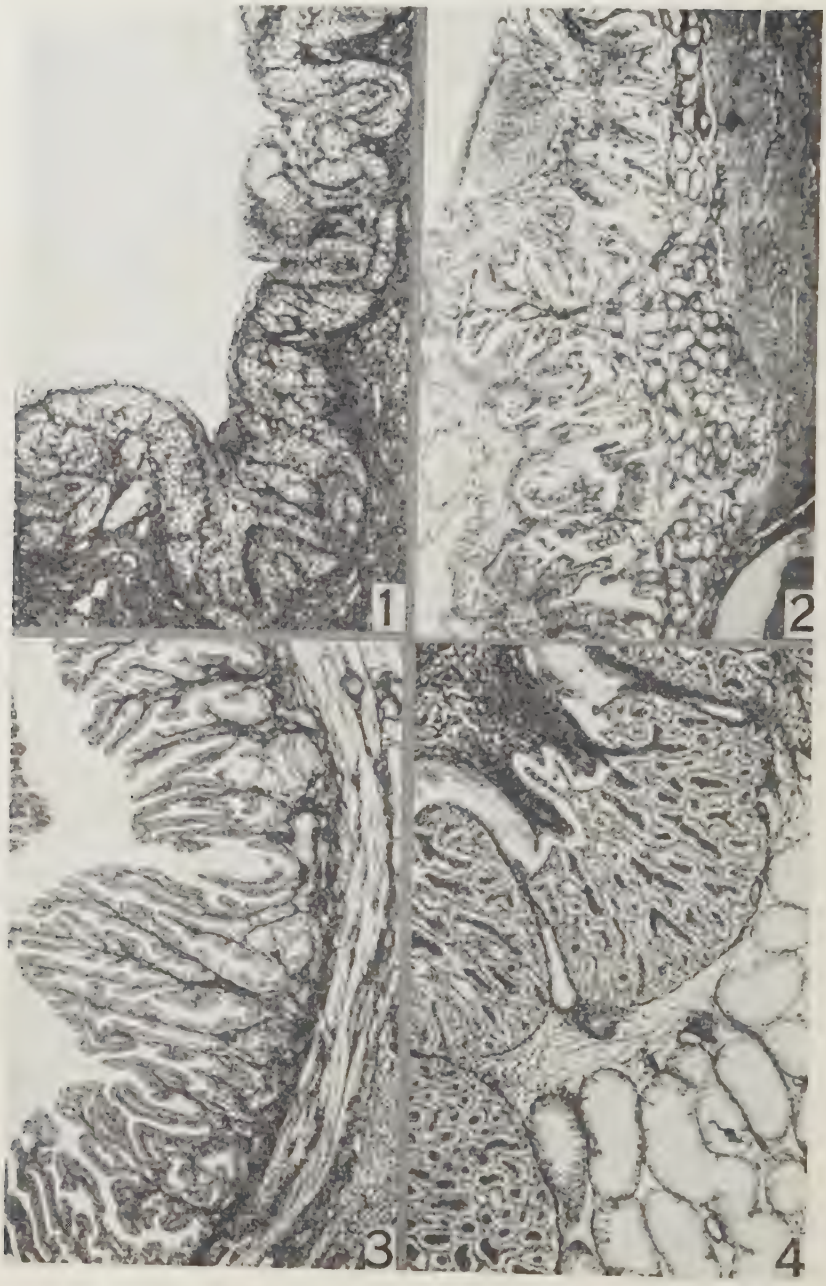

FIG. 1.

Section of the wall of a newly-forming secondary corpus luteum of a pregnant mare (fetal crown-rump length = 10 cm.). The thin layer of granulosa (nearest the cavity) shows degenerative changes, but the underlying theca interna is hyperplastic. × 48.

FIG. 2.

Section of chorion-endometrial juncture from a mare with a 17.5 cm. fetus. The endometrium (right) shows villous proliferation not unlike that produced in the rabbit by injections of progestin (see Fig. 3). × 48.

FIG. 3.

Progestational proliferation in uterus of immature rabbit treated with 18.9 mg. (11.7 gm. fresh tissue) of progestin from secondary corpora of a mare with a 10 cm. fetus. Note formation of vascular, primary, and secondary villi, almost to the exclusion of uterine glands. × 48.

FIG. 4.

Section of chorion-endometrial juncture from a mare with an 83 cm. fetus. The uterine glands (lower right) are hypertrophic and distended. No connection could be seen between them and the lumen. × 48

gestational hormone in the adrenal cortex[9, 10] had not come to our attention until this study was completed.

Through the kindness of Mr. J. H. Rowell, we were able to collect material at his Dublin Canyon abattoir. All of the mares were small, wild mustangs from the southwestern states. The stage of gestation was estimated by the fetal crown-rump length. Ovaries, chorion, endometrium, blood and urine were collected immediately after shooting the animal. Corpora lutea, dissected out of the ovaries, were handled separately in each case, as were the chorions, and separated endometria (see Fig. 2 for the histology of these layers). The blood was citrated, and the plasma used for bio-assay. The urine was collected from the bladder after death.

The tissues and fluids were stored in twice their weight of 95% ethanol, and the finely ground tissues or precipitates extracted with this same ethanol for 4 hours in a Soxhlet. Essentially the same procedure as described by Allen and Meyer[11] was used for extracting progestin, hexane being substituted for petroleum ether. The oestrin fraction was separated for future assay. The weighed crude progestin was dissolved in sesame oil, and usually 90% of an individual yield was injected in 5 daily doses into a single 2-month-old female rabbit previously treated for 6 days with 10 R.U. of oestrin daily.[12, 13] If a strongly positive reaction was obtained, the remaining 10% was tested on another rabbit.

The results included in Table I show that while good yields of progestin were obtained from the corpora, no definite progestational proliferation was observed in any of the rabbits treated with extracts of the other tissues or fluids. This does not necessarily mean that there was no progestin in these tissues. It may have been

9 Engelhart, E., *Klin. Wchnschr.*, 1930, **9**, 2114.

10 Callow, R. K., and Parkes, A. S., *J. Physiol.*, 1936, **87**, 28P.

11 Allen, W. M., and Meyer, R. K., *Am. J. Physiol.*, 1933, **106**, 55.

12 Allen, W. M., *Am. J. Physiol.*, 1930, **92**, 612.

13 McPhail, M. K., *J. Physiol.*, 1934, **83**, 145.

TABLE I.

Assays for Progestin Carried Out on 2-month-old Rabbits Primed for 6 Days with 10 R.U. Oestrin per Day. The Following Equivalents of the Original Tissues or Fluids Were Injected Over a Period of 5 Days.

C. R. Length cm.	Blood cc.	Reaction	Urine cc.	Reaction	Chorion gm.	Reaction	Endometrium gm.	Reaction	C.L. gm.	Reaction
1	225	—	225	—			49	—	4.9	+
2.5	225	—	225	—			29	—	3.6	+
4							48	—	7.6	+
5	225	—	225	—			49	—	7.6	++
8	225	—	54	—			103	—	19.8*	++++
8									1.9	+
10	225	—	225	—	17	—	72	—	11.7*	++++
10									1.1	+
14	225	—	225	—	60	—	69	—	24.3*	++++
14									2.4	+
30	405	—	405	—	216	—	153	—	11.7*	+++
30									1.1	+
40	360	—					310	—	5.8*	++
43	225	—	225	—			159	—	6.9*	+
47	315	—	360	—	342	—	193	—		
52					70	—	40	—	27.0†	+
76	90	—	90	—	90	—	90	—	None	

*Secondary multiple corpora. †Whole ovaries.

there in small traces masked by oestrin incompletely removed in the extraction procedure. The progestin fractions were not assayed for oestrin. Fig. 3 illustrates the degree of uterine proliferation produced with 18.9 mg. (11.7 gm. of fresh tissue) of crude progestin from the secondary corpora of a mare with a 10 cm. fetus. It will be noted that the surface epithelium has proliferated and formed villi quite similar to those produced in the mare's endometrium as the maternal contribution to the equine placenta (Fig. 2). The endometrial glands of the mare appear to be shut off from the uterine lumen, and late in pregnancy when the oestrin titre is still high they become greatly enlarged and dilated (Fig. 4).

As far as we are aware, no one has previously reported on the progestin content of an individual corpus luteum or "crop" of corpora in the case of any animal. Our results show that as little as 1.1 gm. of a pregnant mare's corpus luteum contains an amount of progestin detectable by the immature rabbit test. The assays reported herein, as well as further data obtained from a study of mixed lots of pregnant mare's secondary corpora, indicate that these structures constitute the best "natural" source of progestin yet discovered.

9597

First Estrus in Rats in Relation to Age, Weight, and Length.*

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The experiments here reported represent an attempt to determine whether a relation exists between the growth rate and first estrus in the rat.

It has been common knowledge for a decade that rats and mice may be retarded in growth rate and in size at weaning by the amount of available nourishment. Many breeders have found that optimal conditions of growth and size in an animal colony may be attained by limiting the size of the nursing litter to 5 or 6 animals.

Litters of rats were selected for this study over a period of more

* Aided by a grant from the Committee for Research in Problems of Sex, the National Research Council.

† Guest investigator from the Dept. of Pediatrics, Western Reserve University.

TABLE I.

Group	No. of rats per litter	No. of litters	No. of rats	Weaning Measures, 22d day		Vaginal Orifice—1st Estrus			
				Weight gm. Mean, S.D.	Body Length mm. Mean, S.D.	No. of rats	Age in Days Mean, S.D.	Weight gm. Mean, S.D.	Body Length mm. Mean, S.D.
A	2-3	23	61	41.8±4.8	117.8±8.2	61	46.54±5.6	109.9±27.1	162.0±12.8
B	4-5	15	68*	39.8±6.4	116.7±6.4	75	52.9±12.5	114.8±22.0	166.7±8.88
C	10-11	10	103	22.6±5.19	100.4±8.8	91	77.9±11.8	115.1±19.0	168.8±9.27
D	3-4 yeast fed	15	54	52.5±5	119.0±3	54	41.1±5	127.0±14	166.0±7

*Due to an oversight, weaning measurements were not taken for 7 animals of this group.

than a year. They were taken from every month in the year, but few were studied during July and August. Records were obtained on 286 rats which lived to first estrus. These represented 63 litters. To indicate the random selection of litters for study, the serial numbers of the animals reported range from 13,992, born Sept. 27, 1935, to 16,721, born Oct. 4, 1936. Those of Group D were studied between January and May of 1937.

The sex of the young was noted at birth, and females only were left in each litter. The rats were originally divided into 3 categories; those with 2 or 3 in a litter (Group A), those with 4 or 5 (Group B), and those with 10 or 11 (Group C). The latter represent in each instance 2 litters born the same night, and reared by the mother of one of the litters. Thus half of this group were foster rats. Because of the high mortality of infantile rats under such conditions of crowding, there were in Group C only 7 litters with 10 each alive at weaning and 3 litters with 11 alive at weaning. Subsequent losses reduced this number to 91 at first estrus.

The treatment accorded Group D is detailed below.

All animals were maintained on an adequate diet, used in the laboratory for 8 years. In addition, the nursing females all received special diet (McCollum's). The young were kept together in the same cages after weaning. They were given the usual colony diet adequate for growth, and each was given access to ample food supply after weaning. However, those in larger groups continued to grow at a slower rate than those in the smaller.

Body lengths were taken under deep anesthesia. The measurements include the straight line distance between the nose and the anus. The lengths of the tails were also taken, but were more variable within the group than body lengths.

The establishment of the vaginal orifice was adopted as an endpoint instead of first estrus, as determined by the vaginal smear. Both occurred on the same day in 75% of the rats (Group A 75.4%, Group B 75.0%, Group C 74.7%). With a single examination daily, this might be from one hour to 24 or 30 hours after v. o. In some instances it was felt that the estrous smear was missed entirely, because the animals were given a single daily examination.

A more intensive study was made of the first 111 rats in this series. The animals were autopsied at first estrus and organs were weighed. Nothing of significance was learned from the weights of pituitary, ovary or uterus, and these measurements were discontinued.

It is obvious that in these 3 groups of rats, the only experimental modification was regulation of litter size, in order to affect the

amount of available nourishment. Crowding may have exerted some effect, in addition to the nutrition.

All animals were weaned on the 22d day of life. The data from these observations are summarized in Table I, and graphically presented in Fig. 1. There is nothing new in the observations that litters with 5 or less gave higher average weights at weaning than those with 10 or 11 in a litter. There is no significant difference in size, weight or length, between Groups A and B. Group C is significantly lighter and shorter at this age.

The animals were examined daily from the 30th day of life to the establishment of the vaginal orifice. The earliest age at v. o. was 33 days in several animals in both A and B Groups. The earliest age at v. o. in Group C was 50 days in a single animal, the next at 58 days in a single animal.

The average ages of estrus in Groups A and B, 46.5±5.6 and 52.9±12.5 days, respectively, are not significantly different. In Group C, however, the average age of 77.9±11.8 days is distinctly different.

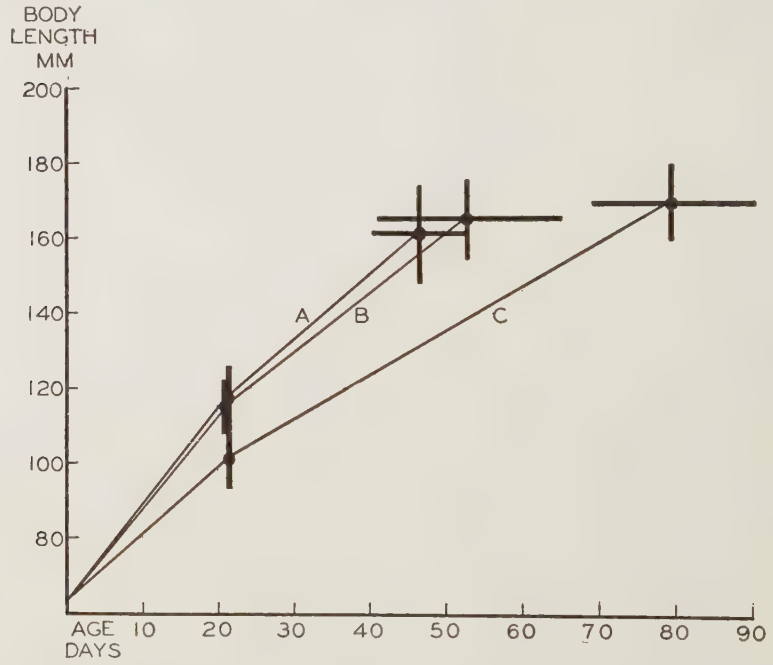

Fig. 1.

Chart showing average body length at weaning, 22 days, of 3 groups of rats, and the body lengths of the same groups at the establishment of the vaginal orifice. Circles indicate average of each group. Horizontal bars are the standard deviations of the ages at vaginal orifice; vertical bars are the standard deviations of body lengths, at weaning, and at v.o. Numerical data from Table I.

Since a slight depression of the growth rate causes a marked delay in the time at first estrus (Group C), it was thought desirable to attempt to accelerate the growth rate beyond that normal for our colony. An experiment employing one type of accelerated growth was conducted by one of us (C.E.Z.) by adding yeast to the diet.

The mother rats (Group D) were fed 2 to 3 gm. of yeast daily during pregnancy and lactation. The females, usually 4, occasionally 3 to the litter, were then fed by medicine dropper 1 to 2 gm. of yeast daily from weaning to the opening of the vaginal orifice.

As is seen from Table I the animals of Group D were heavier (52.5±5 gm.) and longer (119±3 mm.) at weaning than the A Group. They also had the earliest average first estrus, (41.1±5 days). The body weights were greater (129±14 gm.) and highly variable. The body lengths, however, were approximately the same as in the other groups, namely 166±7 mm.

Thus the rats of Group D, in which the growth rate was accelerated by addition of yeast to the diet, grew faster, were heavier, and experienced an earlier first estrus, but they were uniform with the other 3 groups in body length at first estrus.

The significant point brought out by these data is that while estrus occurred at average ages of 41, 46, 53, or 80 days in the 4 groups, ranging from 33 to 105 days, it occurred when the body length reached 160-170 mm., irrespective of the age. It can be believed from these data that age is related only in a secondary degree, within certain limits, to the time of onset of first estrus. At least the only close agreement in this study is between first estrus and body length. Body weights taken as averages for the 3 groups are close together, but the standard deviations show this to be a more variable factor than length. The coefficient of correlation for body weight and length is high for 2 groups: Group A = 0.934; B = 0.909; for Group C = 0.688.

The number of animals in this report is small. For this group, however, it is believed that the conclusions are valid. The body lengths at estrus for the first 111 rats gave results very close to those reported for each group of the completed series. The observations recorded above are not in agreement with those of Asdell and Crowell,[1] although the data are dissimilar. Asdell and Crowell had 2 groups of experimental rats, 21 of which were kept at a greatly reduced body weight by reduction of total caloric intake. The age at first estrus in their rats with most severely depressed growth rates was 357 days. It is difficult to compare our data with that

1 Asdell, S. A., and Crowell, Mary F., *Nutrition*, 1935, **10**, 13.

which Asdell and Crowell present; but from their data we are unable to discover the basis for their statement that "age is a more important consideration than weight in determining the time at which vaginal opening occurs."

Summary. By varying the number of rats in a litter, animals of a slightly retarded growth rate were obtained. Rats raised to the time of vaginal opening, with 2 or 3, 4 or 5, and 10 or 11 in each litter showed vaginal opening at 46.5±5.6, 52.9±12.5 and 77.9±11.8 days, respectively.

While the range of ages at vaginal opening was considerable in the 3 groups, 33 to 105 days, the body lengths showed close agreement. The body lengths in the 3 groups at vaginal opening were 162.0±12.8 mm., 166.7±8.88 mm., and 168.8±9.27 mm., respectively. The body weights were similar in trend, but showed a greater variability than the body lengths.

A small group of 54 rats was maintained at an accelerated growth rate by administration of yeast. First estrus occurred at 41.1 days, but the average body length, like that of the other groups, was 166±7 mm.

9598 P

A Thermostromuhr with Direct Current Heater.*

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From an analysis of the method of measuring blood flow by the Rein[2, 3] thermostromuhr it became evident that the passage of the high frequency current through the intact blood vessel, on which a diathermy thermo-element is placed, results essentially in a localized heating of the wall of the blood vessel. Hence it was of interest to construct a thermostromuhr unit similar to the modified type introduced by Baldes, Herrick and Essex[1] in which a direct current

* Submitted for publication July 3, 1937.

1 Baldes, E. J., Herrick, J. F., and Essex, H. E., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1109.

2 Rein, Hermann, *Z. f. Biol.*, 1928, **87**, 394.

3 Rein, Hermann, in Abderhalden, Emil, *Handbuch der biologischen Arbeitsmethoden*, Berlin, Urban and Schwarzenberg, Abt. 5, Teil 8, 1928-1935, pp. 693-716.

heater replaced the platinum electrodes and likewise was spaced midway between the differential thermocouple. This arrangement of the Rein unit differs somewhat from the thermostromuhr described by Schmidt and Walker[4] in which the hot thermojunction is a silver trough interposed between a direct current heater and the blood vessel.

The arrangement of the direct current heater and the thermocouple in the bakelite block is shown in Fig. 1. The heating unit is made of No. 36 or 38 B. & S. gauge nichrome wire rolled to a ribbon 0.75 mm. to 0.5 mm. wide. This unit consists of a folded loop 'c' soldered to copper wires 'b' (2 strands number 38 B. & S. gauge), the resistance of the loop being at least one ohm. The unit

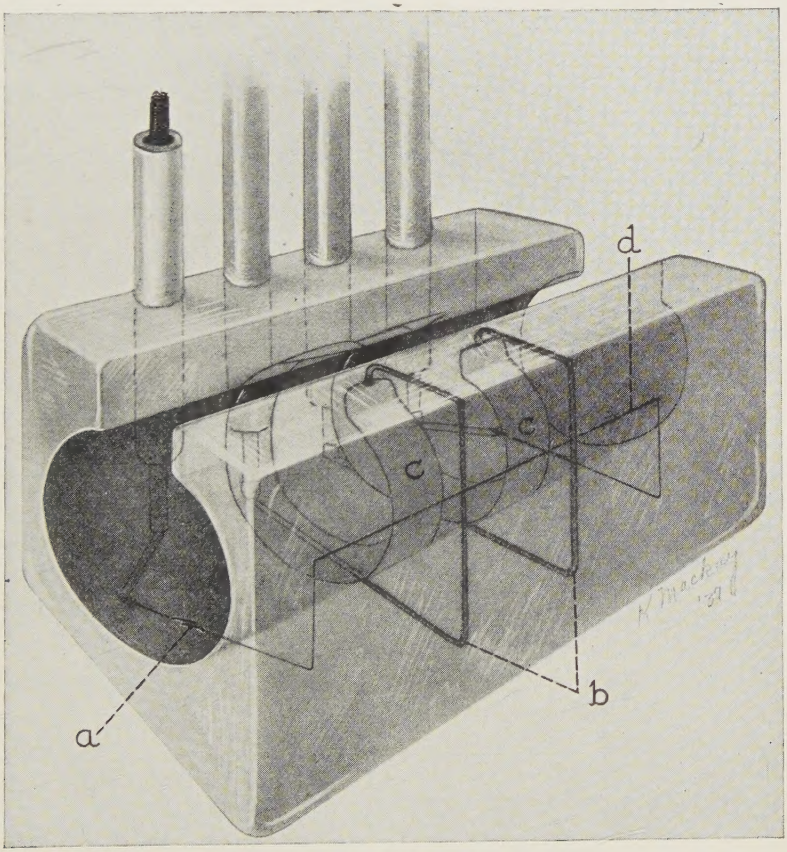

FIG. 1.
A thermostromuhr with direct current heater. The description is in the text.

4 Schmidt, C. F., and Walker, A. M., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 346.

is held in place by bakelite lacquer. The thermojunctions at 'a' are made by soldering copper wires (0.0016 in.) to a constantan wire (0.002 in.) which is embedded in the groove 'd'. The fine copper wires as well as the copper wires attached to the heater are then soldered to braided copper wires (17 strands 0.003 in. tinned copper). All grooves are filled by several applications of bakelite lacquer 3128 or Sterling varnish M-472, each application being followed by suitable air drying and baking. The final step is the insertion of the braided lead wires through rubber tubing 1/32 in. x 1/64 in. and the fixation of the rubber tube in the bakelite block with lacquer.

In using the above type of unit, the heater is connected to an electric circuit containing a 2-volt storage cell or 1.5-volt dry cell, a

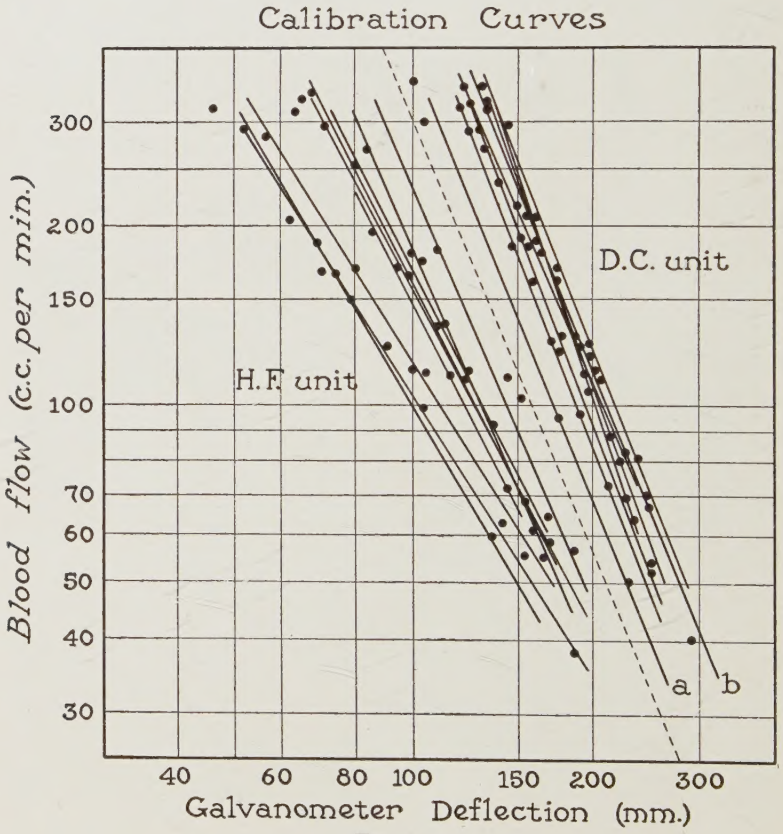

FIG. 2.

Relationship between blood flow and galvanometer deflection for 9 calibrations on a high frequency (H.F.) thermostromuhr and for 14 calibrations on a direct current (D.C.) thermostromuhr. All the curves for the D.C. unit represent "double values" except a and b.

variable resistance and a milliammeter capable of indicating currents up to 500 m.a. The thermocouple is connected directly to a galvanometer, suitable characteristics being one with a low coil resistance, 20 ohms for instance, and a sensitivity of 0.5×10^{-6} volt per mm. for a scale distance of one meter.

A comparison of a series of calibration curves for a high frequency thermostromuhr unit with a series of calibration curves for a similar unit with a direct current heater is shown in Fig. 2. The bore of both units is 2.5 mm. and the differential thermojunctions in each are spaced 1.2 times the diameter from the proximal edge of the heating electrodes or nichrome loop. The heating energy is calculated to be identical for each curve in both series, namely 0.060 calories per second. In the high frequency unit the current was approximately 23.5 m.a. and the high frequency resistance 450 ohms while a current of 475 m.a. was maintained in the direct current heater of 1.1 ohms resistance. In Fig. 2 is shown a series of 9 calibration curves giving the relation between blood flow and galvanometer deflection, with the same high frequency unit on different arteries or veins perfused with defibrinated blood. The other series of curves in Fig. 2 is obtained from 14 calibrations, using the same direct current thermostromuhr on different arteries and veins. A comparison of the 2 series indicates (1) that the direct current unit is as sensitive as the high frequency unit, and (2) that the calibrations check fully as well, if not better, with the direct current thermostromuhr.

Conclusions. A direct current thermostromuhr, similar in construction to the Rein high frequency unit, is described. It is believed that the direct current unit herein described offers all the physiologic advantages of the high frequency type of unit and at the same time introduces a simplified technic for measurement of blood flow.